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Residues of Organochlorine Pesticides and Polychlorinated Biphenyls in Some Commercially Produced Canadian Marine Oils

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Herring oils produced in the late 1960s from Gulf of St. Lawrence fish generally contained 7–17 ppm DDT complex, 0.1 ppm dieldrin, and up to 11 ppm PCBs. In spring, oils were richer in residues than in autumn. Seal oils contained up to 8 ppm DDT complex, and about 4 ppm PCBs, but no dieldrin. Residue levels showed no marked change from the mid-1950s to mid-1960s. North Atlantic whale oils contained up to 40 ppm DDT complex, and (in a few samples only) about 7 ppm PCBs; no dieldrin was found. Some Antarctic whale oils produced about 1950 contained 0.1 ppm DDT complex and no detectable PCBs.

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Les huiles extraites des harengs du golfe Saint-Laurent vers la fin des années 60 contenaient en général de 7–17 ppm du complexe DDT, 0.1 ppm de dieldrine et jusqu'à 11 ppm de PCB. Ces huiles étaient plus riches en résidus au printemps qu'à l'automne. Les huiles de phoque contenaient jusqu'à 8 ppm du complexe DDT et environ 4 ppm de PCB, mais étaient dépourvues de dieldrine. Le niveau des résidus n'a pas changé appréciablement au cours des dix années qui se sont terminées au milieu des années 60. Les huiles de baleine de l'Atlantique-Nord contenaient jusqu'à 40 ppm du complexe DDT et, dans quelques échantillons seulement, environ 7 ppm de PCB; la dieldrine était absente. Des huiles de baleine de l'Antarctique, produites aux environs de 1950, contenaient 0.1 ppm du complexe DDT et n'avaient aucune trace de PCB.

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PESTICIDE residues in plants and animals are concentrated (because of their lipophilic properties) in depot fats. A number of such fats of marine organisms are exploited commercially. In particular, herring, seal, and whale oils are primary raw material for a significant portion of the fats processed in Canada for human consumption (Anon. 1969). There is little published information about pesticide distribution in Canadian Atlantic species. Duffy and O'Connell (1968) and Sprague and Duffy (1971) studied the distribution of DDT and its metabolites in fish and shellfish from the east coast, but did not relate their data to lipid content. The latter authors concluded that residues in Canadian Atlantic species were in the low range of values reported for specimens from more heavily polluted regions, such as other parts of North America, or Europe. Similar inferences can be drawn from a study of North Atlantic seals (Holden and Marsden 1967; Holden 1970a).

This survey of organochlorine residues in some marine oils produced commercially over the past 20 years from species native to the Gulf of St. Lawrence and the western North Atlantic was undertaken to establish the range of residue levels likely to be encountered in raw oils, and to provide a datum base for future studies. For comparative purposes, a number of oils produced elsewhere than the Atlantic Provinces have been included.

Materials and Methods

ORIGIN OF THE OILS

Herring, seal, and whale oils had been collected over the past 20 years. All were samples from commercial production processes, and had been stored in glass bottles with plastic screw tops fitted with liners of pasteboard coated with polyvinyl alcohol or, in the case of herring oils, with corks, under nitrogen at 0 C or below. The latter had been subjected to a preliminary examination shortly after production (Ackman and Eaton 1970).

ANALYTICAL PROCEDURES

Methods used were those outlined in the Pesticide Analytical Manual (1969).

Solvents were either pesticide quality (Fisher Scientific Co.) used without further treatment, or were ACS reagent grade (Fisher) redistilled before use. Florisil® (Floridin Co.) was obtained from Fisher Scientific Co. Pesticide standards were obtained from Applied Science Labs Inc., Supelco Inc., and the Food and Drug Directorate (Canada Department of National Health and Welfare, Ottawa), and were dissolved or diluted in redistilled hexane.

In the early stages of this survey, 3-g quantities of the oils were dissolved in hexane and subjected to partitioning with acetonitrile. The hexane phase was cleaned up on Florisil as described in the Pesticide Analytical Manual (1969). It was found however that acetonitrile partitioning for lipid removal produced spurious peaks on the chromatograms, and subsequently the partitioning step was omitted. In subsequent work samples of up to 0.5 g oil were dissolved in hexane and applied to a 25 g column of Florisil which had been activated at 110 C overnight, slurried in hexane and topped with 1 cm anhydrous Na_2SO_4. Pesticides and polychlorinated biphenyls (PCBs) were eluted with 200 ml each of 6% diethyl ether/hexane (v/v) or 15% diethyl ether/hexane (v/v). This procedure was found to be satisfactory for removal of lipids by adsorption on Florisil. Although the reduction of sample size reduced sensitivity slightly, pesticide levels were sufficiently high to allow analysis by GLC (gas–liquid chromatography).

Portions of the 6% diethyl ether/hexane eluate were dehydrochlorinated (Holden and Marsden 1967) to confirm the presence of *p,p'*DDD and *p,p'*DDT. Production of *p,p'*DDMU, and an increase in the *p,p'*DDE peak after dehydrochlorination were taken to indicate the presence of *p,p'*DDD and *p,p'*DDT, respectively. This procedure was also useful in confirming the presence of PCB peaks with retention times similar to those of *p,p'*DDD and *p,p'*DDT, since the PCBs are resistant to dehydrochlorination. In certain representative samples, PCBs were separated from organochlorine pesticides. Portions of the 6% diethyl ether/hexane eluate from Florisil were taken to dryness and subjected to chromatography on silicic acid-Celite® (Johns-Manville Co.) (Armour and Burke 1970).

As a check on recoveries of residues from the Florisil and silicic acid columns, some samples were "spiked" with standard pesticide mixtures or with PCB (Aroclor® 1254; obtained from Monsanto Canada Ltd.). Blank runs were made from some columns to ensure that interfering materials were not eluted from the absorbents under the conditions employed.

Pesticides were estimated by GLC using the following systems: (1) A Hewlett–Packard 5750 fitted with a ^{63}Ni pulsed electron capture detector (ECD) and 6-ft × ¼-inch coiled glass columns packed with (*i*) 3% SE-30 on 100–120 mesh Gas Chrom Q (operated at 200 C), (*ii*) 6% QF–1+4% SE-30 on 100–120 Gas Chrom Q (operated at 180 C), or (*iii*) 8% QF-1+2% OV-17 on 100–120 mesh Gas Chrom Q (operated at 205 or 215 C). Carrier gas was 5% methane in argon at 40 ml/min; (2) A Packard 7400 fitted with a ^{3}H ECD and 6-ft × ⅛-inch coiled glass packed with 10% DC-200 on 100–120 mesh Anakrom ABS operated at 200 C. Carrier gas was N_2 at 40 ml/min.

All samples were analyzed on both instruments. Injections were made with Hamilton 701-N syringes and peaks were recorded on Moseley or Honeywell strip chart recorders. Peak heights were compared with calibration curves prepared daily by injecting pesticide standards in the 0.1–2.0 ng range. Results are presented as the mean of determinations on both systems. In most analyses on the two systems individual component results were within about 15% of each other.

Results and Discussion

Herring Oils

Organochlorine residue levels in the Gulf of St. Lawrence herring oils are summarised in Table 1. Oils prepared from late winter and spring catches of herring contained 11–17-ppm DDT complex, and those from autumn or early winter catches about 7 ppm. Levels of dieldrin and PCBs were more variable, but if the levels in No. 1 oil are disregarded (since they appear unusually low), dieldrin and PCBs were present at (respectively) about 0.1 and 5–10 ppm in winter and spring oils, and 0.03 and 2–4 ppm in autumn oils.

Seasonal differences in residue levels probably reflect changes in fat content. Fat levels in northwest Atlantic herring generally range from 10–15% wet weight in autumn and early winter down to as low as 3–5% in spring and early summer (Stoddard MS 1968). December and February catches can be considered with spring catches, as fat contents of these fish decline slowly over the winter (Ackman and Eaton 1970). Variation in residue levels in the oils, therefore, probably represents a fairly constant residue concentration in tissue subjected to alterations in fat level by a factor of about three. Levels of total DDT complex in muscle tissue calculated on this basis would be around 0.5–1.0 ppm and proportionately lower for dieldrin and PCBs. These DDT levels are in reasonable agreement with data reported for Gulf of St. Lawrence mackerel (the only other fatty fish from this area studied: Duffy and O'Connell 1968; Sprague and Duffy 1971). DDT complex levels are within the range of 1–25 ppm reported by Dugal (1968) for some freshwater fish oils, but well above the 0.5 ppm quoted by Ruzicka et al (1967) for crude North Atlantic cod liver oil, and by Giuffrida et al (1966) for an unspecified cod liver oil.

Within the DDT complex, only *p,p'* isomers were detected. No peak identifiable unambiguously as *o,p'*-DDT could be detected on any of the three columns employed, but small peaks at the expected position for *o,p'*-DDT were visible in most samples. Probably, however, these were at least partly of PCB origin. The ratio of *p,p'*-DDE: *p,p'*-DDD: *p,p'*-DDT was surprisingly constant in all herring oils examined; expressed as % DDT complex, they were: DDE, 50–59; DDD, 13–18; DDT, 25–33. This probably indicates that all the fish fed at the same trophic level (probably small Crustacea or copepods). Otherwise, the movement of DDT around the food web and its accompanying metabolic and/or photochemical changes would be expected to lead to widely varying DDE: DDD: DDT ratios.

Dieldrin was found in most herring oils at levels of 0.1 ppm or less. This is slightly below the level reported by Ruzicka et al (1967) for crude North Atlantic cod liver oil, and slightly higher than in freshwater fish oils (Dugal 1968).

The occurrence of PCBs in herring oils was not surprising as these compounds have been reported in a variety of samples ranging from wildlife (Bagley et al. 1970) through polluted river water and sewage outfalls (Holden 1970b; Schmidt et al. 1971) to

Table 1. Organochlorine residues in Gulf of St. Lawrence herring oils (as ppm of oil).

Place and date of herring catch	Sample no.	*p,p'*DDE	*p,p'*DDD	*p,p'*DDT	ΣDDT	Dieldrin	PCB
Late winter/spring oils							
S.W. Nfld., Dec. 21, 1968	6	6.37	1.67	3.15	11.19	0.10	8.7
S.E. Nfld., Feb. 11, 1969	7	8.60	1.90	4.05	14.55	0.10	10.9
S.W. Nfld., Mar. 1–4, 1969	8	7.45	1.84	3.87	13.16	0.11	5.5
S.E. Nfld., Mar. 9–19, 1969	9	9.78	2.28	4.59	16.65	0.12	8.1
S.W. Nfld., Mar. 24–28, 1969	10	9.12	2.16	4.45	15.73	0.12	7.6
St. Paul Is.[a], Apr. 17–25, 1969	3	7.53	2.49	5.05	15.67	0.07	10.7
Magdalen Is., Apr. 21–May 12, 1969	4	6.81	2.43	4.48	13.72	0.09	9.9
Bird Rocks[b], Apr. 7–9, 1970	1	8.68	2.57	4.57	15.82	0.03	2.3
Autumn/early winter oils							
Bird Rocks[b], Nov. 19, 1967	5	3.92	0.89	2.61	7.42	ND[c]	1.7
Bird Rocks[b], Oct. 31–Nov. 24, 1969	2	4.29	1.10	1.84	7.23	0.03	3.9

[a]N. of Cape Breton Island.
[b]N. of Magdalen Islands.
[c]ND, not detected.

cardboard boxes (Bailey et al. 1970). Their estimation in herring oils was based on comparison of peaks in residue extracts with Aroclor 1254, which is the PCB mixture most widely used in North America. The GLC "fingerprint" of peaks from herring oil residues other than DDT complex closely resembled that of Aroclor 1254. Generally 8–10 (out of a possible 15) peaks from herring oil coincided with those of Aroclor 1254 on two of the three columns used (Fig. 1a). Some differences in relative peak heights between herring oils and Aroclor 1254 were observed; these probably reflect preferential metabolism of some PCB components (Grant et al. 1971) and so estimates are at best approximate. In some cases, PCB peaks coincided with those of the DDT complex, but the contribution of a PCB component to the pesticide peak height was small ($<5\%$) and so pesticide levels were not corrected.

The possibility that PCBs enter the oils during storage (either from the corks used to stopper herring oils, or from the plastic bottle tops and liners used for the whale and seal oils) was considered. However, analysis of representative stoppers showed that these materials did not contain PCBs. Furthermore, two whale oils (described further below), which had been stored for about 20 years, did not contain PCBs.

Seal Oils

The five oils examined were prepared mainly from blubber of harp seal pups (*Pagophilus groenlandicus*). Results are summarised in Table 2.

No relation between DDT complex level and age or refining was apparent. (Refining here refers to spraying an oil with droplets of warm alkali solution. This process removes free fatty acid, but does not saponify triglycerides, and would hardly be expected to convert DDT to DDE.) The only obvious trend was a slight increase in the percentage of DDE in the complex in more recent oils.

The levels of DDT complex were slightly lower than those reported for hood seal (*Cystophora cristata*) blubber from the Magdalen Islands (10 ppm: Holden and Marsden 1967; Holden 1970a). However, it should be noted that young animals tend to contain lower pesticide residues than do older ones (Holden 1970a). Species differences may also occur.

Dieldrin, reported to be present in blubber from Magdalen Islands hood seals at < 0.1 ppm, (Holden 1970a) was not found in the seal oils studied.

PCBs were identified rather less confidently in seal oils than in herring oils. In general, fewer peaks agreed with Aroclor 1254 standards in seal oils (Fig. 1b) and the fingerprint of PCB in the seal oil chromatogram was much less convincing. However, this may simply represent more extensive metabolism of these compounds, perhaps as a result of movement through more trophic levels than for herring. An estimate of PCB levels in seal oils was made,

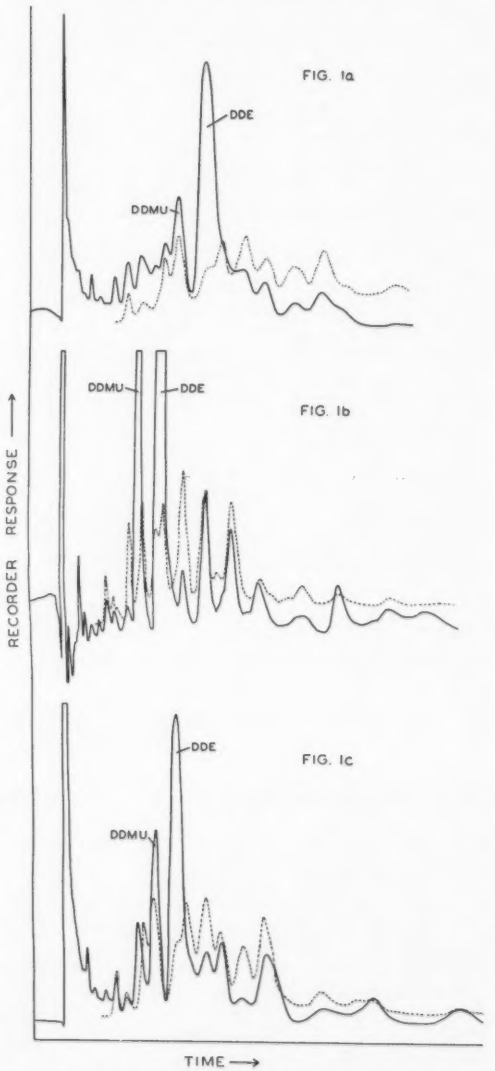

FIG. 1. Chromatograms of pesticide extracts of marine oils, eluted from Florisil with 6% diethyl ether/hexane and saponified as described in text (solid line) and Aroclor(R) 1254 (broken line). Fig. 1a, herring oil No. 1 pesticides, run on QF-1/OV-17 at 205 C; Fig. 1b, seal oil No. 21 pesticides run on QF-1/OV-17 at 215 C; Fig. 1c, whale oil No. 11 pesticides, run on QF-1/OV-17 at 215 C.

based on comparison of peaks that did agree. The ranges (1–5 ppm) are of the same order as those quoted by Holden (1970a) for Magdalen Islands seal blubber.

Whale Oils

Of the Antarctic whale oils, the two oldest (No. 9 and 10, Table 3) contained only traces of pesticide residues. These oils from Blue (*Balaenoptera musculus*) and Sei (*Balaenoptera borealis*) whales were produced by a Japanese firm in the early 1950s, when environmental contamination by pesticides was probably fairly low (DDT came into use in the early 1940s). Even the 1962 sperm whale (*Physeter catodon*) oil contained only traces of residues. In contrast, the two 1966 commercial sperm whale oils (No. 19 and 20) contained appreciable amounts of residues, and here, rather curiously, only DDE and DDD were detected, DDT having presumably been completely metabolised to these compounds.

In recent years analyses of various Antarctic species have shown that this area has suffered some contamination by pesticides (Sladen et al. 1966; Riseborough et al. 1968). However, residue levels reported for marine mammal tissues are generally low (< 1 ppm) and the rather high residue levels, in No. 19 and 20 oils, probably result from the whales feeding in more contaminated areas during migration. It is unfortunate that the detailed history of these oils is not known.

The Hudson Bay beluga (*Delphinapterus leucas*) oil contained fairly low residue levels, and no DDD was detected in the DDT complex. This need not be

Table 2. Organochlorine residues in Nova Scotian produced seal oils (ppm in oil).

Date of origin of oil	Sample no.	*p,p'*DDE	*p,p'*DDD	*p,p'*DDT	ΣDDT	Dieldrin	PCB
1954 (alkali refined)	18	1.30	0.86	0.19	2.35	ND[a]	ND
1961 (alkali refined)	21	3.75	3.02	1.53	8.3	ND	1.4
1961 (crude)	17	3.56	3.07	0.04	6.67	ND	1.8
1962 (crude)	8	4.23	2.42	ND	6.65	ND	3.7
1962 (crude)	15	0.62	0.35	0.06	1.03	ND	1.8

[a]ND, not detected.

Table 3. Organochlorine residues in whale oils (as ppm of oil).

Place and date of whale catch	Sample no.	*p,p'*DDE	*p,p'*DDD	*p,p'*DDT	ΣDDT	Dieldrin	PCB
Antarctic sei ca. 1950	9	0.06	0.11	0.14	0.31	ND[a]	ND
Antarctic blue ca. 1950	10	0.04	0.07	0.07	0.18	ND	ND
Antarctic sperm 1962	23	0.03	0.01	0.03	0.07	ND	ND
Antarctic sperm 1966	19	24.25	10.73	ND	34.98	ND	1
Antarctic sperm 1966	20	19.28	9.27	ND	28.55	ND	1
Hudson Bay Beluga 1966	2	3.69	ND	3.71	7.40	ND	ND
N. Atlantic bottlenose 1962	1	5.38	6.31	ND	11.6	ND	1
N. Atlantic pothead 1962	5	24.25	16.1	ND	40.35	ND	1
N. Atlantic fin 1967	3	2.52	1.72	ND	4.24	ND	ND
N. Atlantic sperm 1967	4	4.96	2.70	ND	7.66	tr	1
N. Atlantic pothead 1967	7	12.05	7.35	ND	19.4	ND	1
N. Atlantic fin 1967[b]	24	14.60	8.45	ND	23.05	ND	ND
N. Atlantic fin 1969	22	18.00	4.27	ND	22.27	ND	1
N. Atlantic fin 1970[c]	11	16.25	8.13	ND	24.38	ND	7.0
N. Atlantic fin 1970[c]	12	24.80	8.53	ND	32.33	ND	5.5
N. Atlantic fin 1970[c]	13	21.10	8.91	ND	30.01	ND	6.8

[a]ND, not detected.
[b]Collected at centrifuge, probably produced from single whale.
[c]Collected from bulk storage tanks; represents composite of several whales.

surprising since both mammalian tissue and fish tissue can metabolise DDT to both DDD and DDE (Menzie 1969) although DDD is probably an intermediate in the ultimate degradation process.

Data for the various North Atlantic whale oils (from fin (*Balaenoptera physalis*), bottlenose (*Hyperoodon* Sp.) sperm and pothead (*Globicephala melaena*) whales) are difficult to interpret. All contained appreciable amounts of residues, although DDT itself was absent and only its metabolites were observed. DDE usually represented the bulk of the DDT group. Dieldrin was present in some oils.

The fin whale generally feeds on krill, mainly *Meganyctiphanes norvegica*, although schooling fish may also be taken, depending on their local abundance (Ackman et al. 1971). *Meganyctiphanes norvegica* taken from the stomach of a female fin whale caught off Nova Scotia in November 1967 had a DDT group level of 0.06 ppm, wet weight (E. R. Kimball unpublished data). This is fairly similar to figures obtained for mixed euphausiids taken at various points in the Gulf of St. Lawrence in mid-1970 (S. R. Kerr and W. P. Vass unpublished data). Comparable data for cephalopods, which are the principal dietary components for sperm, pothead, and bottlenose whales (Sergeant 1962; Tomilin 1967) are apparently not available.

PCBs were confidently detected only in the three 1970 North Atlantic samples discussed further below. Following dehydrochlorination of residue extracts of other oils, a number of peaks corresponding roughly to those found in Aroclor 1254 or 1260 were observed, but only a few of these (generally less than 6 out of a possible 14) coincided precisely with those of standards, and peak height ratios in oil extracts differed markedly from those in standards. We therefore conclude that PCBs are essentially absent from these samples or, if present, have been selectively absorbed, metabolised, or deposited to such an extent that reliable quantitation is impossible.

In contrast, the three 1970 whale oils showed PCB patterns remarkably similar to those in the Aroclor 1254 standard (Fig. 1c). Our estimates of PCB levels in one of these oils (No. 11) was confirmed in another laboratory; results agreed to within 20%. It is not clear whether the PCBs in these oils arose from contamination (during processing, for example) or whether they were deposited as a result of feeding.

Little comparable work seems to have been done on whale depot fat. Wolman and Wilson (1970) found that migrating gray whales from the United States west coast contained, at most, traces of residues; significantly the traces occurred only in the north bound specimens. Sperm whales from the same area contained appreciable residue levels, up to 10 ppm in blubber. Differences between gray and sperm whales were attributed to feeding habits, the former feeding principally in Arctic seas (probably uncontaminated) and the latter feeding in more contaminated areas.

Conclusions

The origin of organochlorine pesticide residues in Canadian Atlantic marine species is not in doubt. From 1950–1965 considerable amounts of DDT (up to 10^6 lb/year) were sprayed on New Brunswick forests to control the spruce budworm; some of this would inevitably leach into the Gulf through the major river systems in the area and into marine life (Fredeen and Duffy 1970). In contrast to the spraying program in New Brunswick, pesticide usage in the other Atlantic Provinces has been relatively light (G. Penney personal communication). With the cessation of the heavy use of DDT in New Brunswick, it would be expected that the Gulf of St. Lawrence would eventually, though slowly, attain a reduced level of persistent organochlorine residues. This optimistic view is offset, however, by another potential source of pesticides, the St. Lawrence River. This drains the Great Lakes Basin, which is heavily contaminated with pesticides and other pollutants (Reinert 1970). Despite the recent ban on DDT usage, the flushing time of this system may be considerable. Our Canadian Atlantic marine life, however, does not seem to be as heavily contaminated with pesticides as does that from areas of heavily industrialised western Europe (Holden 1970a).

The presence of PCBs in many of the samples analysed is no doubt due to the fact that these materials have been widely used for industrial applications since the 1930s, and are now widely distributed in the environment.

Finally, it is important that we point out the actual fate of pesticides in locally produced marine oils. The marine oils for edible use are processed in several ways, often by refining (mild alkali washing to remove free fatty acids) followed by bleaching with activated earths, and are then subjected to catalytic hydrogenation which removes polyunsaturated fatty acids. This is often followed by deodorizing (steam and/or vacuum stripping). The DDT group and dieldrin are effectively removed from vegetable oils treated similarly (Smith et al. 1968) and it seems reasonable to suppose that these particular compounds at least would be removed during processing of fish and marine mammal oils. The effect of processing on PCBs, however, is not known to the authors at the time of writing. Most hydrogenated marine oils are blended prior to packaging in various proportions with vegetable oils. Any levels of organochlorine residues reported in this study are therefore max-

imum values for raw materials, and in no way indicate possible levels in the final edible product.

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Some Relations of Land Drainage, Nutrients, Particulate Material, and Fish Catch in Two Eastern Canadian Bays

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SUTCLIFFE, W. H., JR. 1972. Some relations of land drainage, nutrients, particulate material, and fish catch in two eastern Canadian bays. J. Fish. Res. Bd. Canada 29: 357–362.

A year's record of the carbon content of living and of carbon and nitrogen content of nonliving suspended particulate material from St. Margaret's Bay, Nova Scotia, is presented. From considerations of carbon/nitrogen ratios and nutrient data from other sources, the nitrogen budget is briefly considered. The possible importance of land drainage into the bay leads to some positive correlations between runoff and catch of four commercially important species in the Gulf of St. Lawrence.

SUTCLIFFE, W. H., JR. 1972. Some relations of land drainage, nutrients, particulate material, and fish catch in two eastern Canadian bays. J. Fish. Res. Bd. Canada 29: 357–362.

L'auteur fait connaitre les résultats d'une année d'observation sur la teneur en C du matériel particulaire vivant en suspension et sur la teneur en C et N du matériel non vivant de la baie Sainte-Marguerite, Nouvelle-Ecosse. Se basant sur une analyse des rapports C/N et sur des données provenant d'autres sources sur les éléments nutritifs, il considère brièvement le bilan de N. L'importance possible du drainage des terres dans la baie lui permet d'établir quelques corrélations positives entre le ruissellement et les captures de quatre importantes espèces de poissons commerciaux dans le golfe Saint-Laurent.

Received October 14, 1971

BEGINNING in 1967 with investigations on the primary productivity and nutrient features of St. Margaret's Bay, N. S. (Platt and Irwin MS 1968), a number of studies of various facets of this environment were initiated, among them determinations on the carbon and nitrogen content of the suspended particulate material reported herein. These latter observations led to consideration of some aspects of the nitrogen budget and to the role of land drainage in the bay and at other places in eastern Canada. Because of the resulting correlations of river discharge with some inshore fisheries, it seemed appropriate to present the following preliminary investigations.

Methods

Water samples were collected from September 1968 through August 1969 at approximately 2-week intervals at a depth of 5 m at Station "A" located 1 mile from the western shore of St. Margaret's Bay at 64°02′W. 44°35′N in 58 m of water (Platt and Irwin MS 1968). The water samples were of ½ liter quantity and were strained through 160 μ nylon mesh to eliminate larger plankton organisms. Samples were then filtered through 0.45 μ membrane filters for ATP analysis (Holm-Hansen and Booth 1966) or through 0.8 μ silver filters for particulate carbon and nitrogen determinations (Hewlett Packard CHN analyzer, Model 185). For size fractionation, some samples were first poured through 10 μ nylon mesh or through a 5 μ silver filter with 50% retention effective at about 8 μ and 2 μ, respectively (Sheldon and Sutcliffe 1969). ATP samples were frozen immediately after collection and treatment; samples for particulate carbon were vacuum desiccated until laboratory analysis.

Results

Figure 1A shows concentration with time of living carbon (from ATP Holm-Hansen and Booth 1966) in three size fractions. These estimates were subtracted from total particulate carbon determinations to obtain nonliving carbon (Fig. 1B) for two size fractions. Total particulate nitrogen for two size fractions is also given (Fig. 1C).

Discussion

Of interest is the large proportion of living material in St. Margaret's Bay less than 8 μ in size. Except for the spring bloom, it is obviously important in terms of quantity and accounts for a major portion of the autumn bloom. For the year, the small sizes amount to 60% of the total living particulate material, and, if the spring bloom is neglected, comes

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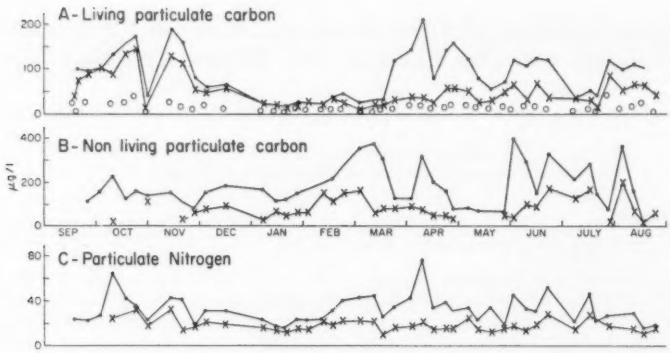

FIG. 1. (A), Living particulate carbon (from ATP); ●—● <160 μ; x—x <8 μ; 0 <2 μ; (B), Nonliving particulate carbon (total less living), symbols as in (A); (C), Total particulate nitrogen, symbols as in (A). All St. Margaret's Bay.

to 67% of the total. Considering faster growth rates for the smaller sizes (Eppley and Sloan 1966) or higher production rates (Watt 1971), this must surely mean that by far the greatest production comes from these small organisms in the bay. A high percentage, annual mean of 68%, of such small living material (5–15 μ) was noted by Riley (1957) in the Sargasso Sea. Concerning the smallest size fraction, less than 2 μ, presumably mostly bacteria, little can be said at this time. Although the annual mean level is 16 μg C/liter which amounts to 19% of the mean annual biomass (higher during the winter), the methods at hand cannot distinguish between the larger sizes and bacteria adhering to particles; thus, the bacterial fraction is likely underestimated.

If one plots total/living particulate carbon against carbon/nitrogen ratios (by weight) for the first half of the year the result is shown in Fig. 2A. As the total/living carbon ratio approaches one as a limit, the C/N ratio approaches 6.1 indicating the C/N ratio of the living material for this time of year. This agrees rather well with phytoplankton chemical analyses made for the spring of 1969 wherein the mean C/N ratio was found to be 5.97 (T. Platt personal communication). The month of March, 1969, was omitted from the plot in Fig. 2A because of the large peak of nonliving carbon preceding the bloom.

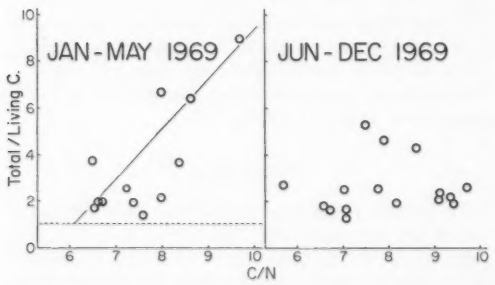

FIG. 2. (A), Total/living carbon vs C/N ratios (by weight) Jan.–May 1969 (r = .731); (B), Same, June–Dec. 1969. All St. Margaret's Bay.

The plot of total/living carbon vs C/N for the remainder of the year was not so satisfactory (Fig. 2B) and the correlation so poor (r = .46) that little information could be obtained other than that the lowest total/living carbon ratios occur at a little above C/N six. The poor correlation was likely due to high and variable quantities of nonliving carbon as may be seen in Fig. 1B. A consideration of total/living vs C/N for sizes less than 8 μ for those months when the small sizes were dominant was not rewarding in terms of good correlation; however, it may be said that the lowest total/living ratios were accompanied by C/N ratios of around 5.5. In all, these results indicate a year-round C/N ratio near six for most living material smaller than 160 μ.

Accepting a C/N ratio of about six for living material, it is possible to examine the C/N ratios for the nonliving portion by subtracting living carbon estimates (from ATP determinations) and the appropriate amount of nitrogen from total particulate carbon and nitrogen, respectively. On this basis, the large peak of nonliving particulate carbon > 8 μ in late February–early March, while rather variable, was accompanied by a mean C/N ratio of 13, suggesting erosion of the large attached seaweeds (C/N 16.6 in August, Mann 1972b) indicated by Mann (1972a) for this time of year. The peaks of > 8 μ nonliving particles in June and July were accompa-

nied by high and variable C/N ratios (14–53) and no conclusion is made as to their origin. However, the mean C/N ratio for the year for the larger nonliving particles was 17.1, which is not far from that of 19.2 for the year for seaweeds (Mann 1972b), again indicating this source as a possible important contributor. Flemer and Biggs (1971) suggest terriginous material as a source of high C/N ratios for Chesapeake Bay where drainage is much more important; we could find no general correlation between runoff or heavy wind and the summer peaks of nonliving material in St. Margaret's Bay.

Nutrients in St. Margaret's Bay

Information on the amount of suspended living carbon and nitrogen in St. Margaret's Bay permits some consideration of nutrients. From inspection of data given by Platt and Irwin (MS 1968) it would appear that nutrient fluxes in the Bay are similar in gross aspect to Long Island Sound, well described by Riley and Conover (1956). In general, nutrients are high in winter, decreasing rapidly with the spring bloom to low levels, and gradually increasing in the deeper layers through the summer while some nutrients are fed into the euphotic zone through a two layered circulation system; recycling or regeneration, at least in Long Island Sound, forms a major supply.

According to Sharaf El Din et al. (MS 1970), a two-layered model may be applied to St. Margaret's Bay, following Pritchard (1967), wherein freshwater runoff is an important circulating factor causing outflow at the surface and replacement by the deeper water of the Bay. From April 1967 through March 1968, this runoff amounted to $1.3 \times 10^9 m^3$, which helped flush the bay at an average of every 40 days or a little more than nine times during the year (bay volume about $4.6 \times 10^9 m^3$), thus moving about 30 times its volume of sea water in the process. This means then, that part of the time, especially during rainy periods, we are looking at a slowly moving segment of the shelf water in the Bay, while during dry spells, St. Margaret's Bay would have some character of its own.

A brief consideration of some aspects of the nitrogen budget for the bay might be helpful. From data given by Platt and Irwin (MS 1968), total primary productionfor 1967 amounted to about 156 g C/m^2year. Using the C/N ratio of six above for living material, this production would require 26 g N/m^2. The other major primary producers are undoubtedly the large attached seaweeds (Miller et al. 1971) which in 1968–69 produced 700 g C/m^2year requiring 36 g N/m^2year at a mean yearly C/N ratio of 19.3 (Mann 1972b). If we are permitted to apply these seaweed figures to 1967 we get a total requirement of 62 g N/m^2year. The mean level of all nitrogen measured in 1967 in the water column (Platt and Irwin MS 1968) was about 2.7 g N/m^2 (2.3 nitrate-nitrite, 0.4 ammonia); thus some 59 g N/m^2year had to be supplied or recycled in some fashion.

Determinations of nutrient content of one of the small rivers (Indian River) entering St. Margaret's Bay are available for April 1969–December 1970 (Water Quality Division, Department of Fisheries and Forestry personal communication). These indicate a mean content of about 0.4 mg N/liter or 400 mg/m^3 (mostly as ammonia). Referring to runoff values quoted above, and the volume of the bay, this would amount to about 7 g N/m^2year if we may apply these runoff analyses back to 1967. The human population around the Bay is small, most of the runoff being derived from forested areas (drainage area 265 mi^2, Sharaf El Din et al. MS 1970), so there is no reason to invoke quantities of nitrogen from sources such as urea.

One of the larger sources of nitrogen in the water column above the bottom was presumably the zooplankton population, which from figures of Platt and Irwin (MS 1968) amounted to a mean annual concentration in 1967 of 2.9 g/m^2 dry weight. The zooplankton was 78% copepods by number and was dominated by the small *Pseudocalanus minutus*. According to R. J. Conover (personal communication) these copepods produce on average, about 11 mg N/g dry weight/day as ammonia, or, applying this to the zooplankton about 12 g/m^3year in the bay. The contribution of the microzooplankton is undoubtedly significant, judging from estimates of relative abundance from Beers and Stewart (1969), but no figures are available nor is the vertical distribution known from St. Margaret's Bay.

Another major source of nitrogen, the in situ production from degradation of organic materials in the euphotic zone, was estimated by the rate of accumulation of nitrogen in deeper water in the summer during periods of relatively low runoff. This amounted to about 0.57 g N/m^2year. Referring this to the upper 25 m, where most of the primary production took place, yielded a supply of about 14 g N/m^2year.

After subtracting nitrogen supplied from runoff, that of the zooplankton (mostly in the upper layers, T. Platt personal communication), and regeneration in the euphotic zone the remaining 26 g N/m^2year needed (neglecting microzooplankton) would have to come from the deeper layers in the Bay as recycled (or regenerated) material or from advection from deeper water outside the bay. There exists some correlation between near surface chlorophyll (from Platt and Irwin MS 1968) and runoff (Fig. 3). It would

be expected that increased runoff would increase the replenishment of nutrients through a two layered circulation, although the importance of wind and tides both in providing energy for the two layered circulation and in producing upwelling directly has not as yet been evaluated. The correlation seems rather good considering the effects of other factors such as temperature, insolation, and winds (Platt 1971). From this brief consideration, it would appear that the nutrient flux stimulated by freshwater runoff may be an important factor in the bay either for recirculating regenerated materials vertically or bringing in and distributing nutrients from the outside.

Adequate statistics for testing long-term effects of runoff in St. Margaret's Bay are lacking. However, with this possibility in mind, it is interesting to look at another situation in eastern Canada. Discharge records are available for the St. Lawrence River, a major drainage system in North America, as well as fishery statistics from the provinces surrounding the Gulf of St. Lawrence into which the river empties. Trites (MS 1971) has calculated that, based on a mean freshwater input of 1.6×10^4 m^3/sec (5.1×10^{11} m^3/year, 15% of this being direct precipitation less evaporation), the Gulf (volume 3×10^{13} m^3) will be flushed with a time constant of 6–8 months depending on assumptions. Neu (MS 1970), working on the St. Lawrence estuary, demonstrated the profound mixing effects produced by the runoff, again based on the two-layered model, wherein the fresh water moves nearly 30 times its own volume, much like St. Margaret's Bay. Thus we appear to have a relatively closed system with a long residence time, but with significant effects of runoff.

According to preliminary studies of Steven (MS 1971), the high nutrient content in the area of the St. Lawrence estuary is derived primarily from upwelling of the deep water of the Laurentian Channel presumably caused by the mixing effects described by Neu (MS 1970) previously mentioned. In terms of the Gulf as a whole, if one applies the nutrient load of the St. Lawrence River (International Lake Erie Water Pollution Board 1969), to yearly freshwater influx and the volume of the Gulf, additions from the river amount to less than one-third of that of St. Margaret's Bay on a volumetric basis.

Mean monthly and yearly discharge records for the St. Lawrence River (from 1922) were obtained from the U.S. Department of Commerce[2] as measured at Lake St. Lawrence (Fig. 4).
These were plotted against records of four species of animals obtained commercially in the Province of Quebec (Fisheries Statistics of Canada). The catch of this province was chosen for geographic reasons; it seemed likely that most landings would come from the Gulf of St. Lawrence. The results are shown in Fig. 5–7. With three out of the four species, catch records seemed to divide naturally into late

[2]National Ocean Survey, Lake Survey Center, Detroit Mich., U.S.A.

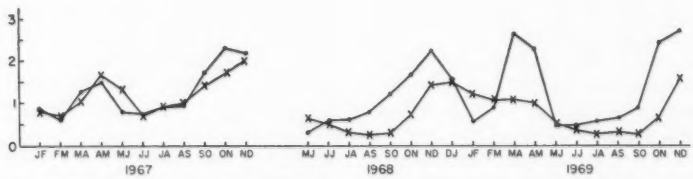

FIG. 3. ● Chlorophyll *a* (μg/l) 1 and 5 m depths, 2-month means from Platt and Irwin (1968); × land drainage (× 10^{3}ft^3/sec) from Sharaf El Din et al. (1970). St. Margaret's Bay (r = .600 for all data, $P < .001$).

FIG. 4. Discharge of St. Lawrence River at Lake St. Lawrence with time, 2-year running averages.

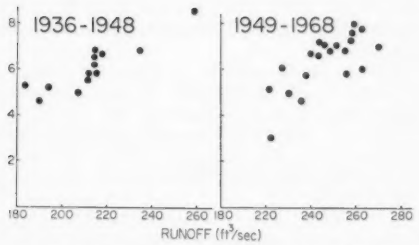

FIG. 5. Lobster pack of Quebec ($\times 10^3$ cases) vs discharge St. Lawrence River ($\times 10^3 ft^3/sec$); 1936–1948 $r = .929$, $P < .001$; 1949–1968 $r = .751$, $P < .001$ no correlation 1929–1935. 6 year slip, 2-year running averages.

and early periods, possibly because of differences in fishing pressure. It is to be noted that the number of years of "slip" required to match catch against runoff, except for haddock, is approximately age at maturity or commercial size (Wilder 1965; Needler and Ingalls 1944; Leim and Scott 1966; and Kohler 1960), indicating runoff is perhaps affecting larval survival or growth. At any rate six out of the eight plots are significant at better than the 1% level by *t*-test[3].

It seems likely that with closer attention to life histories, runoff at critical months (rather than yearly means used here), inclusion of other variables, etc., these relations might be improved and other species considered. Predictive possibilities for inshore fisheries are an obvious and advantageous goal. Further, these relations, if valid, indicate the necessity of considerable planning when contemplating changes or diversions in drainage systems.

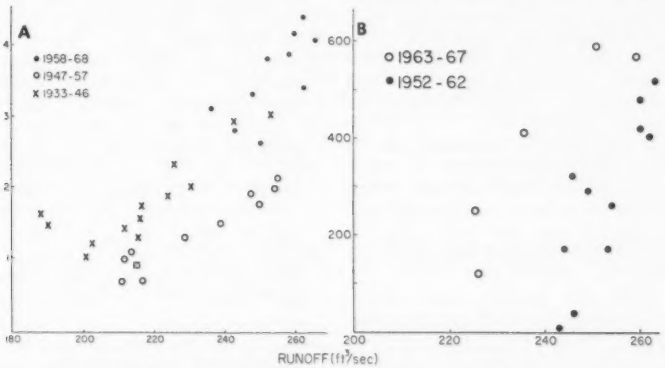

FIG. 6. (A), Halibut catch of Quebec ($\times 10^5$ lb) vs discharge St. Lawrence River ($\times 10^3 ft^3/sec$) 1933–1968 $r = .797$, $P < .001$, 10 year slip, 3-year running averages. (B), Haddock catch of Quebec ($\times 10^3$ lb) vs discharge St. Lawrence River ($\times 10^3 ft^3/sec$); 1952–1962, $r = .934$, $P = .02$; 1963–1968 $r = .773$, $P = .001$, 8 year slip, 3-year running averages.

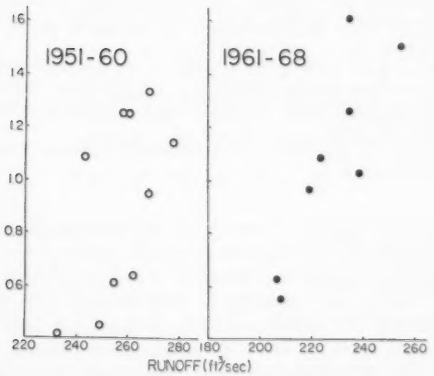

FIG. 7. Clam (soft shell) catch of Quebec ($\times 10^6$ lb) vs discharge St. Lawrence River ($\times 10^3 ft^3/sec$); 1951–1960 $r = .576$, $P < 0.1$; 1961–1968 $r = .853$ $P < .005$. 5 year slip.

In conclusion, the correlations between the physical parameters and biological production do not suggest that control in the natural systems discussed here is simple (see Riley 1949); the geochemical and biological mechanisms considered are demonstrably liable to significant individual variations. Many more extensive observations would be required to establish their real magnitude.

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[3]Comparison of runoff data with quantities of oysters landed from 1928 to 1969 in Prince Edward Island (Morse 1971) using a 2-year running average and a 5-year slip (age at commercial size, R. E. Drinnan personal communication) showed a correlation of 0.656 ($P < .001$).

Acknowledgments

The author is grateful for the assistance of Mrs E. Sutcliffe, Mr A. MacDonald, Mrs A. Orr, and Mrs B. Tiarks, and to Drs G. A. Riley, L. Dickie, R. H. Loucks, and E. M. Hassan for helpful criticism.

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Review of Studies on Feeding of Aquatic Invertebrates Conducted at the Institute of Biology of Inland Waters, Academy of Science, USSR

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MONAKOV, A. V. 1972. Review of studies on feeding of aquatic invertebrates conducted at the Institute of Biology of Inland Waters, Academy of Sciences, USSR. J. Fish. Res. Bd. Canada 29: 363–383.

The main results obtained at the Borok Institute during the last decade are reviewed. Food and methods of feeding by various aquatic invertebrates (Rotatoria, Oligochaeta, Mollusca, Cladocera, Copepoda, Chironomidae larvae), daily food consumption, and assimilation of food have been investigated. Most invertebrates are omnivores although some species live on only one type of food. Daily food consumption changes with food concentration, temperature, and size of consumer. At 15–22 C and a concentration of food close to optimum, mean daily rations of most invertebrates studied usually range from 25 to 100% of body weight. Only in pulmonary Gastropoda and silt-eating Tubificidae does it greatly exceed body weight. In rare cases, at very high concentration of food unusual in nature, the so-called "extra feeding" may take place under experimental conditions. For most invertebrates feeding on natural food at optimum concentrations, index of assimilation varies widely, but rarely exceeds 50%. The assimilability of plant food was 45–55% in the majority of investigated species and appears to be considerably higher when animal food is used.

MONAKOV, A. V. 1972. Review of studies on feeding of aquatic invertebrates conducted at the Institute of Biology of Inland Waters, Academy of Sciences, USSR. J. Fish. Res. Bd. Canada 29: 363–383.

L'auteur expose les principaux résultats d'études effectuées à l'Institut Borok au cours des dix dernières années. Les recherches ont porté sur la nourriture, les méthodes de capture, la consommation quotidienne et l'assimilation des aliments chez divers invertébrés aquatiques (rotifères, oligochètes, mollusques, cladocères, copépodes, larves de chironomides). La plupart des invertébrés sont omnivores, bien que quelques espèces ne se nourrissent que d'un type d'aliment. La consommation quotidienne varie en fonction de la concentration de nourriture, température et taille du consommateur. A des températures de 15 à 22 C et des concentrations de nourriture à peu près normales, la plupart des sujets d'expériences consomment en moyenne chaque jour de 25 à 100% de leur poids corporel. La nourriture absorbée dépasse largement le poids corporel seulement chez les gastéropodes pulmonés et les tubificidés mangeurs de vase. Dans des cas exceptionnels et à des concentrations expérimentales de nourriture très élevées, rarement observées dans la nature, il peut y avoir excès d'alimentation. Chez la plupart des invertébrés se nourrissant d'aliments naturels à des concentrations optimales, l'indice d'assimilation varie beaucoup, mais n'excède que rarement 50%. L'assimilabilité de la nourriture végétale chez la plupart des espèces étudiées est de 45 à 55% et semble beaucoup plus élevée lorsqu'on emploie de la nourriture animale.

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THE efficiency of the processes of biological production is in many respects determined by the completeness and intensity of utilization of primary production by heterotrophic organisms. The study of feeding mechanisms and physiology of digestion of aquatic animals helps to reveal the ways organic matter is transformed and to evaluate the trophic role of animals.

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The food and the methods of feeding by various invertebrates, daily food consumption, and assimilation of food have been investigated at the Institute of Biology of Inland Waters since 1956. Not all groups have been studied to the same extent; the main work has been devoted to the feeding of Crustacea and Mollusca, although we have also studied the feeding of Protozoa and Oligochaeta. The present article reviews the main results obtained at the Borok Institute during the last decade.

Feeding was studied both in nature and in the laboratory. In the laboratory the methods of catching and ingesting food was observed and more complex physiological experiments were made using radioactive tracers (Sorokin 1966, 1968).

Our main indices of the feeding rate and efficiency are: (1) The amount of food assimilated, excluding respiration losses (Ca), is estimated by the formula $Ca = C_r.r_1$. The specific activity of food (C_r) is a ratio of the amount of organic carbon of food (μg C to one pulse of its radioactivity), and r_1 is the radioactivity assimilated by the animal; (2) The index of assimilation (Ca/W) is the ratio of assimilated food (Ca) to the body weight of a consumer (W); (3) The daily ration (C) as a percent of body weight is calculated by the formula:

$$C = \frac{(r_1 + r_2 + r_3) \cdot C_r \cdot 24 \cdot 100}{t \cdot W} \%$$

where: r_2 = radioactivity of CO_2, respired by animals into the medium, r_3 = radioactivity of excrements and liquid excretions, t = the time of experiment (hr); (4) Assimilability (A) is calculated by the formula:

$$A = \frac{(r_1 + r_2) \cdot 100}{r_1 + r_2 + r_3} \%;$$

(5) The coefficient of utilization of food (Ca/C) is assimilability excluding metabolism losses, i.e., the ratio of assimilated food (Ca) to the daily ration (C) excluding metabolism losses.

Protozoa

Only feeding of *Paramaecium caudatum* was investigated. Its food consists of bacteria and various algae (protococcales, diatoms, and blue-green algae). The ability of animals to consume and assimilate forms having firm cellulose cell walls made it possible to assume that *P. caudatum* possesses a powerful enzyme system acting on cellulose (Mordukhai-Boltovskaja and Sorokin 1965). As a rule, smaller food items are better assimilated (Table 1). Apart from algae, which are well assimilated by Infusoria (*Coelosphaerium*, *Aphanizomenon*), some organisms are not used as food (*Microcystis*, two species of *Anabaena*) (Mordukhai-Boltovskaja and Sorokin 1965; Sorokin et al. 1965). The indices of assimilation of *Chlorella*, *Aphanizomenon*, and *Coelosphaerium* given in Table 1 are considerably exaggerated due to methodological faults when determining the carbon content in the bodies of such small animals as *Paramecium*.

Rotatoria

Asplanchna priodonta collected in nature contain various algae, mainly the genera *Pediastrum*, *Ankistrodesmus*, *Eudorina*, *Melosira*, and *Cyclotella*. During algal blooms, the number of species of algae in the intestines of Rotatoria increases, however, remains of *Keratella cochlearis*, *K. quadrata*, *Conochilus* sp., *Bosmina* sp., and copepod nauplii also occur in great numbers (Tribush 1960).

Asplanchna herricki is a filter-feeder-carnivore; strong currents of water caused by the trophi carry food into the mouth opening. The food is then pushed into the stomach by the jaws.

The composition of food of *A. herricki* indicates that these animals are omnivorous. Under experimental conditions Rotatoria survive well only when fed on animal food and quickly succumb when grown on algal cultures.

Table 1. Assimilation efficiency of Infusoria and Rotatoria.

Species	Kind of food	Index of assimilation Ca/W%	Author
Paramaecium caudatum	Bacteria	13	Mordukhai-Boltovskaja and Sorokin (1965)
	Chlorella	74.4	
	Chlamydomonas	32.2	
	Anabaena	0.9	
	Coelosphaerium	67.3	
Asplanchna priodonta	*Aphanizomenon*	77.4	Sorokin and Mordukhai-Boltovskaja (1962)
	Bacteria	0.02	
	Scenedesmus	0.5	
	Protococcum	0.4	
	Anabaena	0.03	
	Chlamydomonas	5.5	
	Bosmina	30.6	

In the opinion of Tribush (1960) algae were chance elements in the food of *A. herricki*. This was confirmed later by special experiments on feeding of both *Asplanchna* species mentioned (Sorokin and Mordukhay-Boltovskaja 1962).

The index of assimilation of various algae and bacteria by these Rotatoria is very low (<0.5%), *Chlamydomonas* being an exception. On the other hand it increases many times and in some cases reaches 30% (Table 1) when Rotatoria are fed on crustaceans.

OLIGOCHAETA

Feeding of Oligochaeta was investigated for species common in Rybinsk Reservoir (*Limnodrilus hoffmeisteri*, *L. udekemianus*, *Isochaetides newaensis*, *Chaetogaster diaphanus*). By dissection the composition of food in *Limnodrilus* was found to be quite uniform (frustules of dead diatoms, mineral particles), while the ratio of various particles in the intestine and the lake sediment is usually the same. This indicates the absence of food selectivity in these worms. But prolonged cultivation (up to 30 days) of *L. hoffmeisteri* on natural silt has revealed food preferences and a clear dependence of composition of food on the quality of mud. On the basis of these experiments it was possible to define a sequence of three phases in the feeding of the worms (Poddubnaja 1961): (1) Valuable food particles, mainly organic, are swallowed; (2) Less valuable mud is swallowed first rapidly, then intensity of feeding declines; (3) The worms stop swallowing the mud that now consists almost exclusively of mineral particles.

Swallowing of mud by Oligochaeta takes place in the following way: the head lobe of a feeding worm elongates and acquires the shape of a wedge. This lobe then expands and bends towards the mouth opening. At the same time the thickened dorsal wall of the pharynx protrudes partly and the concave surface of its ventral wall becomes convex. The mud particles are pushed by the head lobe and are helped by the protruded pharynx (Poddubnaja 1967). Passing of silt through the intestine of *Isochaetides newaensis* and *Limnodrilus hoffmeisteri* lasts 15–30 min.

Although tubificids are capable of active migrations into the silt layers (up to 20 cm), they feed only in a narrow layer under the surface at a depth of 2–5 cm, where highly nutritious compounds are accumulated under the influence of anaerobic decomposition. While feeding at this depth, the tubificids also perform respiratory movements above the sediment surface (Poddubnaja and Sorokin 1961).

The feeding rate of Oligochaeta is rather high. In silt-eating tubificids (*Limnodrilus hoffmeisteri* and *L. udekemianus*) the amount of food consumed daily is 2–8 times the weight of the animal (Table 2). The actual amount ingested depends on the temperature and the kind of silt (Fig. 1, 2). The higher the percentage of easily utilized organic matter in the sediment, the lower the intensity of feeding and vice versa. No daily rhythms in feeding of these species have been found (Poddubnaja 1961).

In contrast to pelophyllic tubificids the pharynx in carnivorous naidids (*Chaetogaster diaphanus*) is a powerful sucking apparatus able to capture actively moving organisms (Poddubnaja 1967). The food approached is felt by tactile sensitive hairs covering the epithelium of the head lobe of the worm. Sucking is brought about by the work of powerful pharynx muscles. The volume of the pharynx cavity increases five times and due to the

TABLE 2. Intensity of feeding of Oligochaeta (18–20 C) (Poddubnaja 1961, 1965).

Species	Kind of food	Ration consumption (C)	
		% of body "wet" weight	(*mg*)
Limnodrilus hoffmeisteri	Silt	703	32.4
L. udekemianus	Silt	745	35.6
Chaetogaster diaphanus	*Bosmina*	27.8	0.7
	Eudiaptomus	23.5	0.6
	Naididae	25.0	0.6
	Chydoridae	22.7	0.5

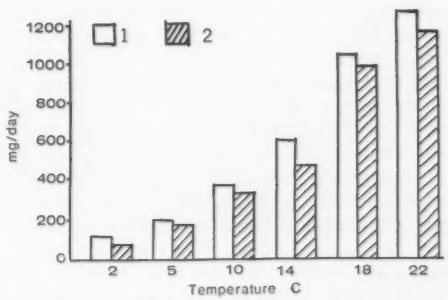

FIG. 1. Feeding rate of *Limnodrilus udekemianus* (1) and *Limnodrilus hoffmeisteri* (2) at different temperatures (Poddubnaja 1961). Abscissa, temperature C; ordinate, weight of excrement in mg per 24 hr.

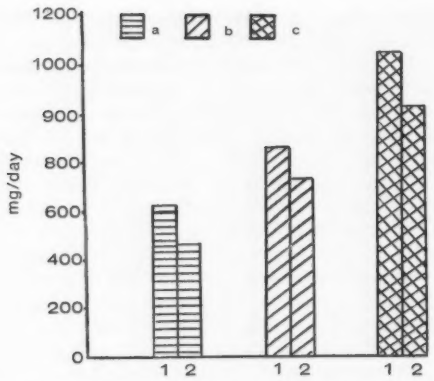

FIG. 2. Feeding rate of *Limnodrilus udekemianus* (1) and *Limnodrilus hoffmeisteri* (2) on different silt (Poddubnaja 1961). a, grey silts; b, intermediary silts; c, peaty silts. Ordinate, weight of excrement in mg per 24 hr.

decrease of pressure inside it, the prey is sucked in with some water. The inflow velocity of the water reaches approximately 1.5 mm/sec, enough to catch prey 7 mm long and 0.9 mg weight from a distance of 7 mm. The width of prey may be up to 2.5 mm, i.e., 10 times the diameter of the unexpanded pharynx. In *C. diaphanus* the period of digestion depends on temperature and may last from 2.5 to 7 hr (Poddubnaja 1965).

Various small invertebrates: Rotatoria, Cladocera, Ostracoda, Copepoda, some naidids, and mites serve as main food for *C. diaphanus*. Usually chydorids are preferred food and are especially abundant in areas populated by the worms (Poddubnaja 1965).

The intensity of feeding changes noticeably with age. In young individuals the value of daily food consumption is low (9% of the body wet weight). During the period of intensive growth when the worms increase in weight and size, the daily ration of *Chaetogaster* also increases and reaches 32% of body wet weight (average –27%). The feeding rate of old and large individuals decreases and the daily ration does not exceed 20%. Daily food consumption is not dependant on the specific nature of food (Table 2), but is closely associated with temperature (Poddubnaja 1965).

HIRUDINEA

In the Rybinsk reservoir the genus *Herpobdella* is well represented. Intestinal contents of *H. nigricolis* (Brand), *H. octoculata* (L.), and *H. testacea* (Sav.), reveal the diversity of food of the *Hirudinea* (two species of Oligochaeta, three species of Cladocera, two species of Copepoda, and 17 species of Chironomidae larvae were recorded in the food). Usually the food composition of *Herpobdella* reflects the composition of benthic fauna at that time (Luferov 1963). In a water body the Hirudinea start to feed late April to early May at a water temperature of 5–9 C. The most intensive feeding is in August. In October it stops completely as temperature drops to 2–3 C.

The *Herpobdella* are carnivorous, actively catching their prey and swallowing it whole. On the second or the third day after hatching from a cocoon young leeches start feeding intensively. They react only to moving prey (Luferov 1963). The amount of food consumed daily changes notably with age but also depends on the type of food (Table 3).

GASTROPODA

The Prosobranchia are typical detritophages, having no mandibles. The intestines of *Viviparus contectus*, *Bithynia tentaculata*, *Valvata piscinalis*, and *V. pulchella* may contain sand grains, silt, unicellular algae (diatoms, green algae), bacteria, and tissues of higher plants. *Bithynia tentaculata* and *Valvata piscinalis* thrive in aquaria and reproduce when fed on suspended algae (Tsikhon-Lukanina 1961a). A similar species, *Valvata pulchella*, feeds mainly with the help of a radula but is capable of filtering (Tsikhon-Lukanina and Sorokin 1965a).

Planorbiidae have a weak radula, as in Prosobranchia, yet they also have mandibles, with which they take in soft food such as detritus, epiphitic algae, and well-putrefied animal food. Higher vegetation is taken in reluctantly. Generally Planorbiidae prefer to take food which requires the least effort (Tsikhon-Lukanina 1965). Limnaeidae, in contrast to Planorbiidae, have a powerful mouth

TABLE 3. Feeding of *Herpobdella nigricollis* (18–22 C) (Luferov 1963).

Weight of *Herpobdella* (*mg*)	Ration in % of body "wet" weight	
	Oligochaeta	Tendipes
0.6	246.9	–
1.2	215.0	177.4
2.5–3.0	168.9	134.7
40–45	26.0	20.2
55–60	21.0	13.1
65–70	32.0	9.2
95–100	19.9	11.0

apparatus which helps them ingest both hard and soft food equally well. The intestines of *Limnaea* collected in nature contained chiefly higher plant tissues. Unicellular algae also occurred in small amounts. Apart from this *Limnaea* may eat their own eggs, those of amphibians, and the bodies of dead animals (Tsikhon-Lukanina 1965).

The feeding rate of Gastropoda on plant and animal food is inversely proportional to their weight. Plant and animal foods are usually consumed in equal amounts (Table 4, Tsikhon-Lukanina 1965).

Prosobranchia feed more slowly than other groups probably because they can filter-feed and consequently are not so active as Pulmonata. The feeding rates and metabolism of *Limnaea* are higher. These molluscs are characterised by their greater activity caused by their feeding behaviour (scraping of plant tissues, periphyton, etc). All Pulmonata expend extra energy ascending to the surface to fill the lung with air (Tsikhon-Lukanina 1965). The amount of food consumed by some Pulmonata is closely associated with food concentration (Tsikhon-Lukanina 1965; Tsikhon-Lukanina and Sorokin 1966). In *Coretus corneus* and *Physa fontinalis*, for instance, mean daily food consumption usually increases with increased food concentration up to a certain limit and then falls (Table 5).

In Prosobranchia the feeding rate is also closely associated with the concentration of food particles (Tsikhon-Lukanina 1961b). Ingestion rates increase with increased food concentration, while filtering speed is inversely proportional to food concentrations.

In *Bithynia tentaculata* we have an analogous picture but only at concentrations higher than 5×10^3 cells/ml. (Table 6).

Temperature also influences feeding rate of molluscs, but not as markedly as in Oligochaeta. The difference in mean daily rations in some Gastropoda (*Planorbis planorbis*, *Anisus vortex*) at temperatures of 10 and 30 C does not exceed 12%, and in others the difference may be still less (Tsikhon-Lukanina 1965).

Valvata pulchella utilizes dead blue-green algae best. *Protococcales*, especially *Chlorella*, are utilized

TABLE 4. Feeding of Gastropoda (19–21 C) (Tsikhon-Lukanina 1965).

Species	Weight (*mg*)	Ration, % of body dry weight: plant food	animal food	avg
Prosobranchia				
Valvata piscinalis	0–50	117	153	135
Bithynia tentaculata	0–50	123	121	122
	0–100	63	48	55
Viviparus contectus	0–50	124	64	99
	51–100	81	58	70
	101–300	37	23	30
Pulmonata				
Coretus corneus	0–50	171	163	167
	51–100	113	124	118
	101–300	47	34	40
	301–500	25	23	24
Limnea stagnalis	0–50	350	347	349
	51–100	–	331	331
	101–200	–	227	227
	201–500	–	134	134
Radix ovata	0–50	400	445	422
	100–200	–	165	165
Galba truncata	0–100	–	242	242
	101–200	–	220	220
Physa fontinalis	0–50	424	395	410

TABLE 5. Intensity of feeding (daily ration in % of body dry weight) of Mollusca under different concentrations of food (Tsikhon-Lukanina 1965).

Weight of food (*g*)	No. of observations	Number of mollusks: *Coretus corneus* 1	3	9	*Physa fontinalis* 1	3	9
0.08	24	14.0	10.0	4.4	33.0	11.0	1.8
0.12	18	26.3	12.0	5.3	66.0	22.0	4.0
0.30	18	94.0	54.3	17.5	400.0	130.0	44.0
0.80	18	170.0	23.3	10.7	295.0	70.0	26.0

TABLE 6. Dependence of intensity of feeding of Gastropoda on concentration of *Scenedesmus* at 18–20 C. Each rate is that of one individual (Tsikhon-Lukanina 1961a).

	Bithynia tentaculata		*Valvata piscinalis*	
Concn of food ('000 *cells/ml*)	Feeding rate ('000 *cells/hr*)	Filtration rate (*ml/hr*)	Feeding rate ('000 *cells/hr*)	Filtration rate (*ml/hr*)
0.5	18.8	42.0	14.2	35.0
1.0	53.1	71.0	21.0	25.0
5.0	223.1	88.0	127.0	31.0
10.0	314.6	42.0	100.0	11.0
25.0	410.0	20.0	320.7	14.0
50.0	1288.7	31.0	960.6	22.0
100.0	980.4	11.0	585.4	5.0

least efficiently. Diatoms (dead and living Nitschia) are utilized equally well by *Valvata* (Tsikhon-Lukanina and Sorokin 1965b). The maximum value of the coefficient of utilization (up to 18%) is noted when *Valvata* are fed dead cells of *Aphanizomenon*, and the minimum value when fed with *Microcystis* (Sorokin et al. 1965). Utilized food usually comprises only a small part of that consumed (Fig. 3).

The coefficient of food utilization in *Valvata pulchella* does not usually exceed 50% when feeding on various types of food (Tsikhon-Lukanina and Sorokin 1965b).

BIVALVIA

Of the Bivalvia, the Sphaeridae are investigated best of all. Under natural conditions their food consists of detritus, bacteria, algae, and probably Protozoa. In *Pisidium* filtered water enters the mantle cavity through the opening in the mantle. This opening is usually in the anterior part of the body where the foot protrudes. Species of *Sphaerium* and *Musculium* may suck in water through the opening when the branchial syphon is closed. These molluscs are capable of filtering suspensions using either the syphon or the opening in the mantle, or both at the same time (Mitropolskij 1966 a, b; Fig. 4). The syphons and the mantle edges forming the opening strictly regulate water inflow. When a suspension of nutrient detritus is added to the aquarium, the molluscs direct the openings of their syphons towards the food source. On the other hand, introduction of toxic substances causes feeding to cease instantly. Many *Sphaerium* and *Pisidium* draw small Infusoria into the mantle cavity and reject big ones when feeding on ciliated plankton. *Sphaerium scaldeanum*, *Pisidium henslovanum*, and *P. ponderosum* are capable of collecting food from substrate surfaces. Such collecting is carried out by a ciliated part of the foot (Fig. 5). This mode of feeding secures normal growth of the molluscs and is apparently widespread (Mitropolskij 1970).

The same kind of food is of different nutritional value for different Sphaeriidae: the mean linear surplus growth of *Musculium lacustre* on bacterial food is 22, on *Chlorella* culture 35, and on *Ankistrodesmus* 22.5%. Linear surplus growth is the difference between the body length at the end of experiment and that at the beginning, expressed as

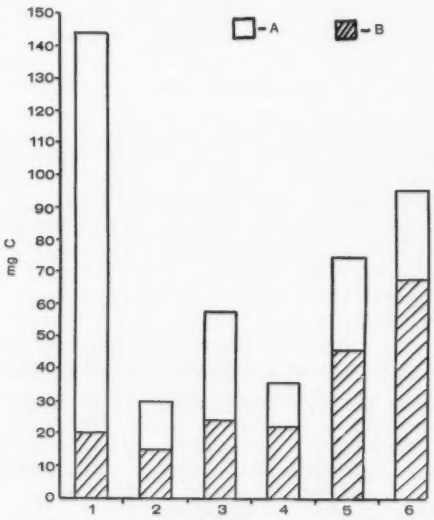

FIG. 3. Intensity of feeding and assimilation by *Valvata pulchella* (Tsikhon-Lukanina and Sorokin 1965a). 1, *Chlorella* (alive); 2, *Scenedesmus* (alive); 3, *Scenedesmus* (dead); 4, *Nitzschia* (alive); 5, *Nitzschia* (dead); 6, *Elodea* (alive, ground to powder). A, daily ration (C) in μg C; B, values of food assimilation (Ca) in μg C.

percent of initial size. In *Pisidium subtruncatum* and *P. obtusale* fed on the above food, the mean linear surplus growth is 14, 18, and 20%, respectively (Mitropolskij 1966a).

Filtration rate of *Sphaerium corneum* is inversely proportional to concentration of food particles (Table 7).

The intensity of feeding of *Dreissena polymorpha*, which is widespread in the Rybinsk reservoir, is also dependent on the concentration of food particles (Mikheev 1966).

When feeding on *Chlorella* cultures of various concentrations, the speed of filtration of water by molluscs ranges from 5 to 50 ml/hr (Fig. 6). The intensity of feeding of *Dreissena* changes with age (Sorokin 1966). The mean daily ration of young molluscs is higher than that of old ones. Assimilability of food, on the other hand, does not change appreciably (Table 8).

Cladocera

The most complete investigation of feeding rate for a cladoceran has been done on *Daphnia longispina*. Its mouth apparatus is equipped with a very fine filter. Bacteria, minute particles of detritus, cells of *Chlorella*, small solitary algae, and also colonies of blue-green algae consisting of two or three cells are swallowed. Larger colonies of diatoms and blue-green algae, which are abundant in the Rybinsk Reservoir, are rejected (Manuilova 1958a). The bulk of food of *D. longispina* in the Rybinsk Reservoir consists of bacteria (Manuilova

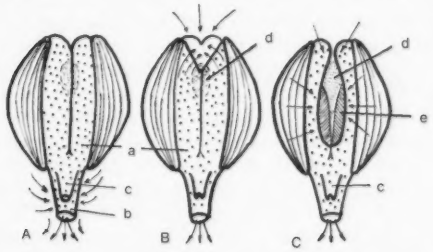

Fig. 4. Position of the mantle margins of *Musculium lacustre* when feeding (Mitropolskij 1966a). A, margins of mantle are closed, siphons are opened; B, mollusc filters under the water surface; water flows through the "window" of the mantle, the gill siphon is closed; C, the same as for "B", the mantle margins are not closed, the window is large. a, brinks of mantle; b, gill siphon; c, anal siphon; d, foot; e, gills.

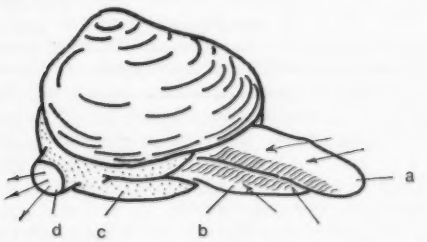

Fig. 5. Movement of water along the groove of the foot of *Pisidium casertanum* (Mitropolskij 1966a). a, foot; b, groove; c, margins of mantle; d, anal siphon.

Table 7. Feeding of *Sphaerium corneum* on *Chlorella* (15 C) (Mitropolskij 1966a).

Concn of algae		Filtration rate
'000 cell/ml	*biomass g/m³*	*(ml/animal/hr)*
7.3	0.3	64
25.0	1.0	44
50.0	2.1	25
100.0	4.2	11
300.0	12.6	11
1000	42.1	4.7
3000	126.3	1.2

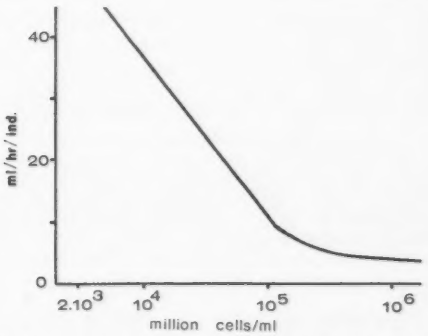

Fig. 6. Filtration rate of *Dreissena* (Sorokin 1966). Abscissa, concentration of *Chlorella* (cells/ml); ordinate, filtration rate (ml/hr, 1 ind.).

Table 8. Intensity of feeding of *Dreissena polymorpha* on bacteria (5 million cells/ml) (Sorokin 1966).

Size of animals *(mm)*	Filtration rate *(ml/animal/hr)*	C % of body carbon content	A %
28	30.5	1.2	43
22	29.5	1.6	46
17	32.0	3.02	57
8	18.0	12.4	49

1958a, b, 1959), but many algae, including some blue-green, compose part of its ration and possess a high nutritive value.

The intensity of feeding of *D. longispina* expressed as index of assimilation is subject to considerable fluctuation and is closely associated with the concentration of food particles (Monakov and Sorokin 1960a, 1961). At low concentrations of bacteria the index of assimilation is proportional to food concentration. The index reaches maximum value at about 2×10^6 cells/ml, and remains practically constant at higher concentrations (Fig. 7). Indices of assimilation for *D. longispina* (Fig. 8) and *D. pulex* (Fig. 9) fed on algae show a similar relation to food concentration. The indices of assimilation for these species fed on algae are rather close (Monakov and Sorokin 1961; Sorokin 1966).

The water filtration rate of *Daphnia* is inversely proportional to food concentration (Table 9).

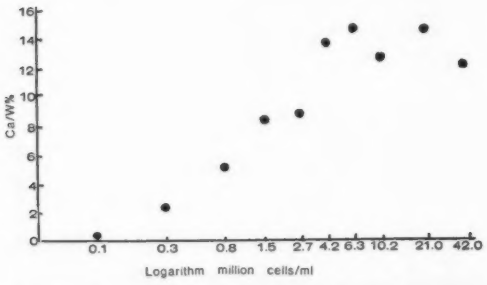

FIG. 7. Dependence of assimilation of food by *Daphnia longispina* on concentration of bacteria (15 C). Abscissa, concentration of bacteria (logarithm million cells/ml) ordinate, index of assimilation (Ca/W%).

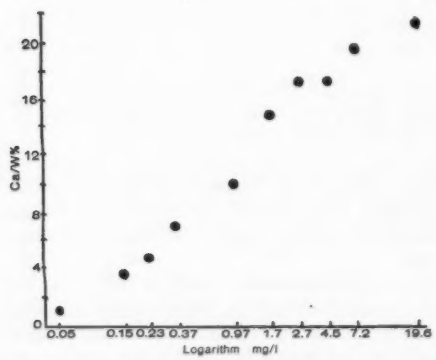

FIG. 8. Dependence of assimilation of food by *Daphnia longispina* on concentration of algae (*Chlorococcum*, 15 C). Abscissa, biomass of algae (logarithm g/m^3); ordinate, index of assimilation (Ca/W%).

At various food concentrations the values of daily food consumption, assimilation, and the coefficient of its utilization change considerably (Table 10). When concentration of *Chlorococcum* rises (Table 10), filtration rate of *Daphnia* decreases and daily ration increases. The amount assimilated and the index of assimilation (Ca/W) are maximal at concentrations of algae close to those found in nature. At higher concentrations of food the daily ration increased but not the assimilation (Ca). As a result the coefficient of utilization of (Ca/C) diminishes (Fig. 8, 9, Table 10).

Daphnia and apparently other Cladoceran filtrators are capable of regulating the intensity of filtration depending on concentration of food. The

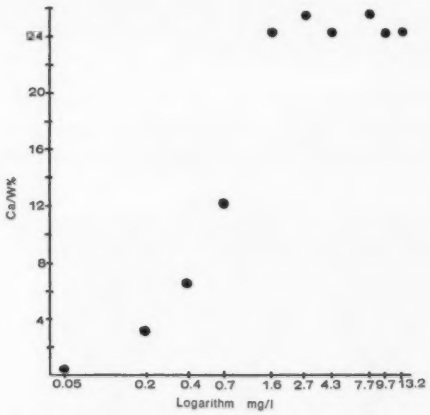

FIG. 9. Dependence of assimilation of food by *Daphnia pulex* on concentration of algae (*Chlorococcum*, 15 C). Abscissa, biomass of algae (logarithm g/m^3); ordinate, index of assimilation (Ca/W%).

TABLE 9. Filtration rate by *Daphnia longispina* (15 C) (Monakov and Sorokin 1961).

Food	Food concn		Filtration rate (*ml/animal/day*)
	million cells/ml	*g/m^3*	
Bacteria	2.4	1.9	5.4
	4.8	3.9	4.0
	9.6	7.7	2.8
	79.0	63.0	0.2
Chlorococcum	*thousand cells/ml*		
	5.5	0.7	16.7
	10.8	1.3	17.2
	22.5	2.8	7.05
	42.1	5.2	3.7

TABLE 10. Intensity of feeding of *Daphnia* on *Chlorococcum* (15 C) (Monakov and Sorokin 1961).

	Concn of food					C		
	1000 cells/ml	g/m³	Ca (μg C/animal/day)	Ca/W%	Filtration rate (ml/animal/day)	in μg C	(%) body carbon	$\frac{Ca}{C}$ %
Daphnia pulex (16 μg C/animal)	2.4	0.3	1.63	10.0	64.0	4.9	30.6	33.2
	6.3	0.8	2.84	17.7	24.0	6.0	37.5	47.5
	15.4	1.9	3.90	24.3	15.8	8.8	55.0	44.2
	33.0	4.1	4.27	26.6	9.5	13.1	81.0	32.6
	53.0	6.6	4.08	25.5	5.4	12.2	76.0	33.5
	116.0	14.3	3.60	22.5	3.0	20.2	126.0	17.8
D. longispina (6.2 μg C/animal)	8.4	1.2	1.0	14.4	14.0	4.1	66.0	24.5
	25.0	3.1	1.2	17.4	7.0	6.2	100.0	19.0
	59.0	7.4	1.5	21.7	4.1	9.2	149.0	16.3
	92.0	11.2	1.2	17.4	2.9	12.2	197.0	9.8

ability to regulate speed of filtration is naturally manifested more distinctly at food concentrations found in nature. At higher food concentrations (higher than 4–6 g/m³ in the case of *Chlorococcum*), decreases in filtration take place at slower rates. The ration continues to grow but not at the expense of an increase of (Ca) assimilation, which takes place at extremely low concentrations but as a result of falling of the (Ca/C) coefficient. Finally, at very high food concentrations these crustaceans can no longer reduce the speed of filtration enough to limit the food intake to the normal daily ration. Under such conditions there may be "extra feeding", i.e., the ration increases but assimilability decreases. The crustaceans have no time to digest and assimilate this extra food. This phenomenon apparently occurs very rarely in nature (Monakov and Sorokin 1961).

Feeding of other cladoceran filtrators was not studied in detail. When solving various methodological problems, however, or when determining nutritive value of various kinds of food, data of some interest were obtained. It was established that *Sida crystallina*, *Simocephalus vetulus*, and *Ceriodaphnia quadrangula* that are widespread in the littoral zone of the reservoir assimilate blue-green algae *Aphanizomenon* and *Coelosphaerium* quite well (Sorokin et al. 1965). In contrast, the assimilability of some Protococcales (*Chlorella*) by Crustacea appeared to be low even though daily rations were high (Table 11).

It is established that the main source of food for many Chydoridae is natural detritus, on which they thrive and reproduce (Smirnov 1962a, b, 1965).

The best studied carnivorous cladoceran is *Polyphemus pediculus*. The food cannot be studied by dissecting the gut of the species caught in nature, since the contents of the intestine are a homogenous mass (Butorina 1965). *P. pediculus* actively pursues its prey, seizing it with powerful pectoral legs and forcing it into the food chamber which is a groove running along the intestine from the first to the fourth pair of legs (Fig. 10, Butorina 1965). Once in the food chamber, the prey is enclosed by tightly folding the pectoral legs. The mandibles grind the food and pass it to the upper lip, then to the opening of the oesophagus that sucks in ground food by rhythmic contractions (Butorina 1965; Butorina and Sorokin 1966).

Although *P. pediculus* feeds mainly on animal food, algae and detritus are occasionally ingested (Table 12). The size of food taken is to some extent determined by the distance between the open catching legs and by the depth of the food chamber (Butorina 1970). The dimensions of food taken by

TABLE 11. Intensity of feeding and assimilation efficiency of some Cladocera on *Chlorella*.

Species	C % of body carbon	A %	Author
Bosmina longirostris	96.0	43.0	Sorokin 1966
Daphnia longispina	93.0	42.5	"
Simocephalus vetulus	108.0	31.0	"
Simocephalus exspinosus	59.0	46.1	"
Bosmina coregoni	–	41.7	Semenova 1972

newly hatched individuals and males may range from 4 to 440 μ. These are mainly Protozoa, Rotatoria, and sometimes first instar Chironomid larvae. Apart from the animals mentioned, Copepod larvae, small Cladocera, and the young of *Polyphemus* make up the food of adult females. Dimensions of food range from 10 to 520 μ.

Species eaten by *P. pediculus* in different seasons of the year are to a great extent dependent upon the concentration of food. Because of a high food demand it prefers abundant prey species (Butorina 1970). If different food objects occur in equal concentrations, however, they prefer certain species.

Extremely high feeding activity of *Polyphemus* is also demonstrated by the absence of an adaptation

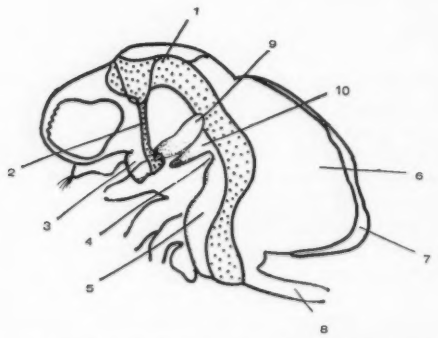

FIG. 10. A longitudinal section of *Polyphemus pediculus* (Butorina 1967). 1, intestine; 2, gullet; 3, upper lip; 4, food chamber; 5, base of food chamber; 6, brood case; 7, outward membrane of brood case; 8, caudal stalk; 9, mandible; 10, maxilla.

period (they start feeding immediately after being placed in an aquarium), by fast filling of the intestine (from 1 to 5 min) and by great speed of digestion (20–25 min) (Butorina and Sorokin 1970). When feeding on a preferred food (e.g. *Bosmina longirostris*, or its own young) that is assimilated effectively, daily food consumption increases proportional to increases in food concentration up to certain limits and then sharply drops (Fig. 11). A similar relation is observed when *Polyphemus* is fed on loricate Rotatoria (*Keratella*) that are assimilated much less efficiently than *Bosmina*, and on first instar Chironomid larvae. At very high concentrations of food assimilation decreases by three times (Butorina 1971).

The index of assimilation when feeding on favourite food increases to an optimal value with increased concentrations of food and then falls sharply (Fig. 12). In other words, the Ca/W value does not form a plateau as in many other Cladocera.

Leptodora kindtii and *Bythotrephes longimanus* like *Polyphemus* tear up the prey with their mandibles, and then suck it in. Studies of their feeding by gut analysis are therefore difficult, since gut contents usually form a homogenous, indefinite mass (Mordukhai-Boltovskaja 1960). *Leptodora* and *Bythotrephes* are very catholic feeders. They eat most planktonic Crustacea, Rotatoria, and planktonic stages of Chironomid larvae.

Preferred prey seldom have hard shells or carapaces. Smaller and slower organisms are also preferred. For example, adult *Leptodora* and *Bythotrephes* rarely use chydorids and cyclopoids as food. They prefer *Polyphemus*, *Bosmina*, and *Ceriodaphnia* (Mordukhai-Boltovskaja 1958). In spring, adult *Leptodora* and *Bythotrephes* feed

TABLE 12. Feeding of *Polyphemus pediculus* on various prey (18–20 C) (Butorina and Sorokin 1966).

Prey	Condition of prey	Ca (μgC/animal/day)	Ca/W%
Chlorella	alive	0.016	0.1
Scenedesmus	"	0.11	0.7
Nitschia	"	0.051	0.3
Asterionella	"	0.026	0.2
Microcystis	"	0.005	0.03
Aphanizomenon	"	0.013	0.1
Detritus of *Elodea*	putrefied	0.015	0.1
Bacteria	alive	0.030	0.2
Bosmina	"	2.280	15.5
Asplanchna priodonta	"	0.580	3.9
Conochilus	"	3.860	26.3
Brachionus	"	1.148	7.8
Paramaecium	"	3.370	22.9

mainly on Rotatoria and Copepoda, and in summer, fed on Cladocera (Mordukhai-Boltovskaja 1960).

The feeding rate of carnivorous crustaceans is rather high (Table 13) and depends greatly on temperature. The optimal value is 18–20 C (Mordukhai-Boltovskaja 1960).

Copepoda

All Copepoda may be divided into three groups on the basis of feeding habits: filtrators, carnivores, and those scraping food from the surface of substratum. Some species are capable of filtering suspended particles and hunting actively (mixed type of feeding). The composition of food of some Copepoda is shown in Table 14.

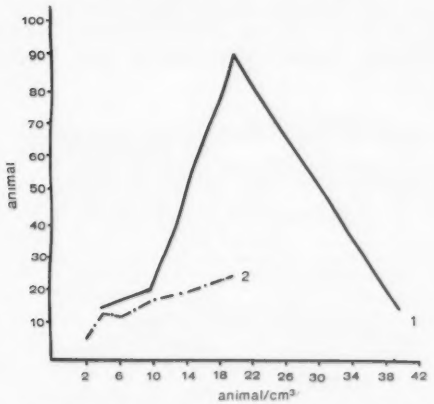

Fig. 11. Feeding rate of *Polyphemus pediculus* (Butorina 1971). 1, *Bosmina longirostris* 2, young stages of *Polyphemus*. Abscissa, concentration of food (ind/cm³); ordinate, daily ration (ind).

Most Cyclopoida, especially large individuals, are active predators, seizing prey when they collide with it. The main catching organs are two pairs of maxillae and maxillipeds. Mandibles are used mainly to tear up and grind the carapaces of prey. Algae, large Protozoa, and small Rotatoria are usually swallowed whole. Some cyclopoids and their naupliar stages can use bacteria (Monakov and Sorokin 1960b). Some larger objects, for example, planktonic Crustacea, chironomid larvae, and Oligochaeta are first torn up and then sucked in or swallowed (Monakov 1959; Monakov and Sorokin 1959b).

Among cyclopoids studied *Macrocyclops distinctus* stands apart; it feeds on detritus and dead animal corpses (Monakov 1963).

The majority of Calanoida including the Diaptomidae feed mainly by filtration. Fast vibration of the second antennae and the mandibular palps causes water currents to pass over the bristles of the second maxillae and maxillipeds, where suspended particles are caught. This suborder also includes carnivorous crustaceans and omnivorous species. The main catching organs of carnivorous Calanoida are the second maxillae and maxillipeds (Monakov 1967, 1968). Usually the mechanism of feeding is reflected in the structure of the mouth apparatus (Fig. 13).

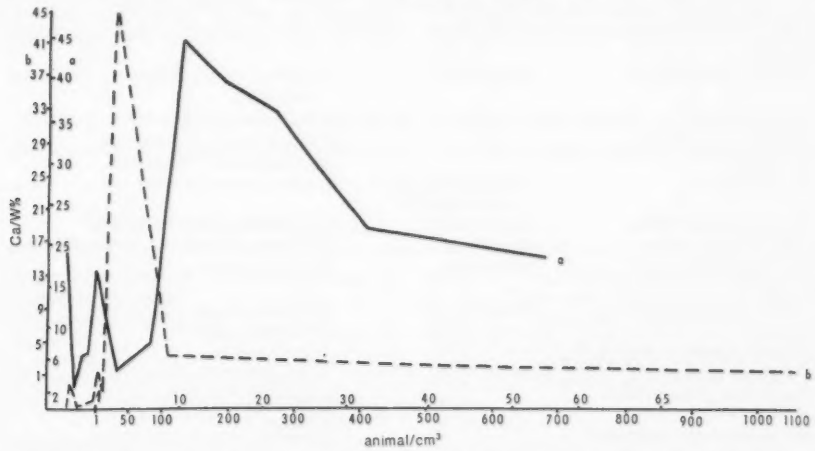

Fig. 12. Assimilation efficiency of *Polyphemus pediculus* (Butorina 1971). Abscissa, concentration of food (ind/cm³); ordinate, index of assimilation (Ca/W%). a, *Ceriodaphnia*; b, *Bosmina*.

Bryocamptus minutus (Harpacticoida) has food habits similar to small cyclopoids of the genus *Microcyclops*, crawling along the substratum and feeding on Protozoa and Rotatoria. As for *Polyphemus* the food is chosen for its accessibility (its size, speed, and the toughness of body coverings). Small animals with delicate coverings are preferred (Monakov 1959, 1963; Monakov and Sorokin 1971a). The structure of their mouth parts corresponds to available food and food preference. Thus, *Eudiaptomus gracilis* and *E. graciloides* are coarse filtrators incapable of catching solitary bacterial cells (Malovitskaja and Sorokin 1961a, b). *Neutrodiaptomus angustilobus* may filter particles as small as 3 μ (Paveljeva and Sorokin 1971). *Eurytemora velox* prefers animal food but also uses algae: it does not eat animals < 3 or > 200 μ (Monakov and Sorokin 1971a).

The feeding rate of carnivorous Copepoda is high. Daily consumption of various food objects by cyclopoids and calanoids at food concentrations usually observed in nature ranges from 35 to 86%, averaging 52.8% of body weight (Table 15). The mean daily ration of omnivorous crustaceans (similar to *Eurytemora velox*) is also rather high.

At the opposite extreme, daily consumption of the filtrator *Eudiaptomus* is low, only 4% of body weight at a concentration of algae from 1 to 4 mg/l. These crustaceans are characterized by low metabolism levels (Malovitskaya and Sorokin 1961a).

TABLE 13. Feeding of *Leptodora kindtii* and *Bythotrephes longimanus* on planktonic Crustacea (Mordukhai-Boltovskaja 1960).

	Leptodora (L=4–5 mm) Ration (*C*)			*Bythotrephes* (L=1.5–2 mm) Ration (*C*)		
Temp (*C*)	Maximum number prey eaten	in μg	% of body wet weight	Maximum number prey eaten	in μg	% of body wet weight
10–15	16	160	21.3	9	90	15.0
15–20	25	250	33.3	27	270	45.0
20–25	30	300	40.0	25	250	41.6

TABLE 14. Feeding of Copepoda.

Species	Method of feeding	Preferred foods
Cyclopoida		
Mesocyclops leuckarti	carnivorous	Infusoria, young stages of Crustacea, Rotatoria
Cyclops vicinus	"	planctonic Crustacea
Macrocyclops albidys	"	Entomostraca, Oligochaeta
M. fuscus	"	Entomostraca, Oligochaeta
M. distinctus	scraping food from substratum	detritus, animal corpses
Acanthocyclops viridis	carnivorous	Oligochaeta, Entomostaraca
Microcyclops bicolor	mixed type	Rotatoria, Protozoa, animal corpses
Eucyclops macruroides	mixed type	Protozoa, algae
Calanoida		
Eudiaptomus gracilis	filtrators	planktonic algae
E. graciloides	"	planktonic algae
Acanthodiaptomus denticornis	"	algae, bacteria
Eurytemora velox	mixed type	algae, Protozoa, Rotatoria
Heterocope appendiculata	"	planktonic Crustacea, algae
H. saliens	"	planktonic Crustacea, algae
Hemidiaptomus amblyodon	"	bacteria, algae Entomostraca
Harpacticoida		
Bryocamptus minutus	scraping food from substratum	detritus, animal corpses

Daily food consumption is much influenced by temperature and food concentration. With higher temperature the daily ration of carnivorous cyclopoids increases, reaching a maximum at 20 C, then decreasing with further temperature increases (Monakov 1959).

In *Eudiaptomus* the ratio of consumed food to body weight increases from 2.2 to 12.5% with increased food concentration (Malovitskaya and Sorokin 1961a).

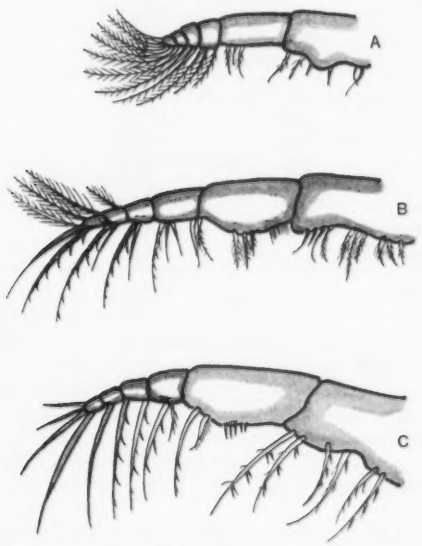

FIG. 13. Structure of maxillipeds of *Calanoida* used for different types of feeding. a, the limb for filtration; b, for filtration and raptorial feeding; c, for raptorial feeding.

Under conditions of very high concentrations of food particles (rarely met with in nature), *Eudiaptomus* may exhibit "extra feeding" similar to filter feeding Cladocera. But in this case the increase of daily ration goes on very slowly due to the decrease in water filtration speed (Malovitskaya and Sorokin 1961a).

A good indicator of the usefulness of feeding is the index of assimilation (Ca/W); at the same time this value characterizes the nutritive value of different kinds of food for invertebrates. Assimilation indices of different kinds of food by Copepoda are shown in Table 16. As may be seen, the assimilability of different prey varies greatly.

For carnivorous crustaceans the amount assimilated ranges from 5 to 55%. In omnivorous forms (*Eurytemora, Heterocope*) fed on algae, assimilation is extremely low (0.3–0.4%). By comparison in typical filtrators (*Eudiaptomus, Acanthodiaptomus*) feeding on algae and bacteria, the value of Ca/W is high and ranges from 6 to 53%.

There is a definite correlation between the index of assimilation and food concentration for all copepods. With increased concentrations of food the effectiveness of feeding rises, finally becoming stable or falling at very high concentrations (Fig. 14). In all cases the assimilation index is stable at food concentrations usually found in nature. This is confirmed by experiments with *Acanthodiaptomus denticornis*, which frequently populates small pools, where the concentration of food is especially high. The index of assimilation for this species decreases, when the biomass is 16–30 g/m^3 (Fig. 14, Monakov 1971).

TABLE 15. Intensity of feeding of Copepoda at natural concentrations of food (15–20 C).

Species	Kind of food	Ration C% of body weight	Author
Mesocyclops leuckarti	*Entomostraca*	63.0	Monakov and Sorokin 1959a
Acantocyclops viridis	"	64.0	Monakov and Sorokin 1959a
Macrocyclops albidus	"	86.0	Monakov 1963
M. fuscus	"	33.0	Monakov 1963
Cyclops vicinus	"	37.0	Monakov and Pugatsheva 1971
Eurytemora velox	*Chlorella*	61.6	Monakov and Sorokin 1971a
Heterocope appendiculata	*Entomostraca*	46.0	"
	Scenedesmus	0.8	"
Hemidiaptomus amblyodon	*Entomostraca*	41.0	"
Eudiaptomus gracilis E. graciloides	*Chlorococcum*	4.0	Malovitskaja and Sorokin 1961a

TABLE 16. The using of various kinds of food by Copepoda at 15–20 C.

Species	Food	Concn	Ca/W%
Macrocyclops albidus	*Ceriodaphnia*	2–3 g/m³	88.0
Eucyclops macruroides	*Stephanodiscus*	4 g/m³	2.0
	Infusoria	800 ind/ml[a]	18.0
Eucyclops serrulatus	*Staurastrum*	4 g/m³	21.0
Cyclops vicinus	*Bosmina longirostris*	4 g/m³	55.0
	Infusoria	150 ind/ml	16.2
	Staurastrum	4 g/m³	0.44
Cyclops kolensis	*Entomostraca*	2 g/m³	16.0
Acanthocyclops viridis	*Entomostraca*	2 g/m³	11.7
Acanthocyclops vernalis	*Bosmina longirostris*	3 g/m³	29.0
	Infusoria	400 ind/ml	54.4
	Chlorella	4 g/m³	0.03
Mesocyclops leuckarti	Infusoria	150 ind/ml	44.0
M. oithonoides	Infusoria	150 ind/ml	32.0
Microcyclops bicolor	Infusoria	400 ind/ml	48.0
	Anabaena	4 g/m³	5.0
Eudiaptomus gracilis	*Chlorococcum*	4 g/m³	6.0[b]
Eudiaptomus graciloides			
Hemidiaptomus amblyodon	*Bosmina*	1–2 g/m³	7.2
	Scenedesmus	3 g/m³	5.8
Acanthodiaptomus denticornis	*Scenedesmus*	4 g/m³	43.0
Heterocope appendiculata	*Bosmina longirostris*	1–2 g/m³	34.0
	Scenedesmus	4 g/m³	0.3
Eurytemora velox	Rotatoria	1.5 g/m³	40.0
	Chlorella	4 g/m³	0.4
Bryocamptus minutus	*Scenedesmus*	3 g/m³	20.0

[a]ind = individual animal.
[b]Malovitskaja and Sorokin 1961a.

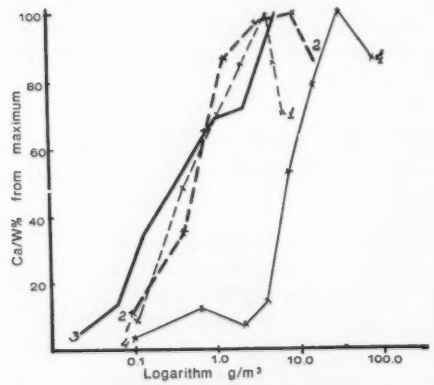

FIG. 14. Assimilation efficiency *Copepoda* 1, *Heterocope appendiculata* (food, *Ceriodaphnia*); 2, *Mesocyclops oithonoides* (food, Infusoria); 3, *Neutrodiaptomus angustilobus* (food, *Stephanodiscus*); 4, *Acanthodiaptomus denticornis* (food, *Stephanodiscus*). Abscissa, logarithm biomass (g/m³); ordinate, index of assimilation (Ca/w% of maximum)

OSTRACODA

Ostracoda are widespread in the Rybinsk Reservoir especially in shoal areas. *Dolerocypris faschiata*, *Cypridopsis vidua*, and *Notodromas monacha* dominate. The gut contents of *D. fasciata* form a brownish dark mass consisting of tissues of higher plants and remains of various algae. These include benthic and epiphitic species from the littoral regions of the reservoir and the cysts of *Dinobryon*, *Mallomonas*, *Achnanthes lanceolota*, and *Cosmarium* sp. (Luferova and Sorokin, 1971). *D. fasciata* feeds mainly by gnawing the periphyton off macrophyte leaves and scraping the growth from submerged substrata. The crustaceans are also capable of ingesting lumps of detritus loosened with the claws of the walking leg and the second antennae. *Cypridopsis vidua* feeds in approximately the same way (Luferova and Sorokin 1970). *N. monacha* may also filter suspended algae and feed on neuston. When feeding on neuston they attack the ventral side of the shell to the surface film, moving along it to collect floating food particles (Luferova 1970).

TABLE 17. Intensity of feeding and assimilation efficiency by Ostracoda (Luferova and Sorokin 1970, 1971).

Kind of food	*Dolerocypris fasciata*		*Cypridopsis vidua*	
	C% of body carbon	A%	C% of body carbon	A%
Chlorella (alive)	117.7	44.2	104.8	69.2
Potamogeton (fresh)	3.8	72.7	3.7	88.1
Fungi	373.2	62.7	157.1	63.1
Detritus of blue-green algae	67.8	72.5	–	–
Detritus of *Chlorella*	10.6	27.4	–	–
Detritus of *Potamogeton* with bacteria and Protozoa	–	–	211.9	84.6
Detritus of *Potamogeton* (sterile)	–	–	104.8	61.5
Mollusc excrement	13.5	29.0	128.6	43.7
Yeasts	166.6	66.9	–	–
Bacteria of the surface film	–	–	116.7	42.8

The feeding of Ostracoda is very diverse. They feed on macrophytes, corpses, excrement of some animals, detritus, algae, fungi, and Protozoa (Luferova 1970). Feeding rate is dependent to a great extent on the character of food. Maximum daily rations are noted when these crustaceans feed on fungi, yeasts, or *Chlorella*. Minimum ones are noted when they feed on fresh *Potamogeton* (Table 17) which is better assimilated. The degree of utilization of detritus depends upon age and character of the detritus.

The assimilability of animal detritus is, as a rule, higher than that of plant detritus, in some cases reaching 85% (Luferova 1970).

CHIRONOMIDAE LARVAE

Much information has accumulated on the food and feeding of Chironomidae larvae (Table 18).

In no other group is there so much variation in the methods and mechanisms of feeding in morphologically close species. As an example we can cite data on the feeding habits of *Parachironomus* larvae, united into the pararostratus group. The first is *P. vitiosus*. As in most other larvae feeding by filtration, it builds up by secretion of salivary glands a trapping conical net. Forcing water actively through the loricae, the larvae catch food particles (algae, detritus) with the net. Every 2–3 min it eats the catch together with the net and builds up a new one, which takes only 15–20 sec. *P. arcuatus* is capable of actively catching passing animals (mainly protozoa) as well as filtration, using macrophytes as food and collecting masses of algae. Finally, *Parachironomus kuzini* larvae, inhabitants of temporary spring pools, are active predators. When young (first and second instars) they feed on different algae and detritus. In the third and at the beginning of the fourth instar, they start feeding exclusively on amphibian eggs and Chironomidae larvae (Shilova 1968).

The feeding mechanism of *Odontomesa fulva* larvae is very peculiar. Having swallowed water, the larva forces it back out by a strong contraction of the intestine. The food-particles contained in water are trapped by numerous bristles of the mouth apparatus (Shilova 1966).

Among the larvae of the genus *Cryptochironomus*, the majority of which are carnivorous, there are forms having mouth parts adapted for collecting sediment. The upper jaws are extraordinarily long and directed forward and downwards. The lower lip with its wide middle tooth acts like a spade in collecting food (Shilova 1960). Carnivorous chironomid larvae (*Ablabesmyia monilis*) attack their prey actively and pierce it with the mandibles. The contents of the prey are sucked into an expanded pharynx with a help of the glossa (Luferov 1957).

The main composition of the food of chironomid larvae is given in Table 18. In the detritophage *Chironomus plumosus*, the percentage food composition is closely associated with food in the habitat. Thus, the contribution of algae, bacteria, organic and mineral substances is different in different parts of the reservoir (Margolina 1961). The composition of food of some carnivorous chironomid larvae under natural conditions is to a considerable degree determined by the presence of food objects. *Procladius* and *Ablabesmyia* larvae eat larvae of silt-eating Chironomidae readily and those of Oligochaeta and Crustacea reluctantly.

Larvae of the first instar prefer smaller forms (Luferov 1961). Carnivorous larvae of Tanypodinae are able to survive for a long time without food, and their resistance to famine is higher in the later instars. In some cases fourth instar *Procladius* larvae are capable of surviving for 2 months without food at 20 C (Luferov 1961).

Carnivorous larvae feed intensively. The average daily food consumption is sometimes twice the body weight and greatly depends on temperature (Table

TABLE 18. Feeding of Insecta larvae.

Species	Method of feeding	Prevailing food	Author
Chironomidae			
Chironomus plumosus	Mixed	blue-green, diatomea, protococcal algae, detritus, bacteria	Shilova (1955a); Margolina (1961); Sorokin and Meshkov (1958, 1959)
C. pilicornis	"	algae	Shilova (1955a)
C. tentans	"	algae	Shilova (1955a)
C. antracinus	"	detritus	Isvekova and Sorokin (1969
Cryptochironomus ussoriensis	carnivorous	Tubificidae	Shilova (1959)
Endochironomus albipennis	filtrators	planctonic-algae	Lukanin (1962, 1963)
Glyptotendipes glaucus	"	"	Lukanin (1962, 1963)
G. imbecillis	"	"	Lukanin (1962, 1963)
Harnischia burganadzeae	scraping food from substratum	detritus, algae	Shilova (1960)
Lipiniella arenicola	scraping food from substratum	algae	Shilova (1963)
Parachironomus vitiosus	filtrators	seston	Shilova (1965a)
P. arcuatus	mixed	seston, macrophyts, Protozoa	Shilova (1968)
P. kuzini	carnivorous	amphibia's eggs	Shilova (1968)
P. longiforceps	scraping food from substratum	periphyton	Shilova (1968)
P. biannulatus	filtrators	seston	Shilova (1968)
Stictochironomus crassiforceps	scraping food from substratum	detritus algae, bacteria	Shilova (1965b)
Orthocladiinae			
Odontomesa fulva	passive filtrators	different algae	Shilova (1966)
Cricotopus silvestris	scraping food from substratum	blue-green, diatoms, protococcales, dead plankton	Rodova and Sorokin (1965); Shilova (1955b); Sorokin et al. (1965)
Tanypodinae			
Ablabesmyja monilis	carnivorous	Chironomidae larvae, Chydoridae	Luferov (1957)
Procladius nigriventris	"	"	Luferov (1956)
P. choreus	"	"	Luferov (1961)
Anatopinia plumipes	"	Chironomidae larvae	Luferov (1956)
Trichoptera			
Phryganea grandis	scraping food from substratum	macrophytes	Smirnov (1962a)
Diptera			
Nematocera	scraping food from substratum	detritus, algae, fungii	Smirnov (1961)

TABLE 19. Average daily rations (% of body wet wt) of carnivorous *Pelopiinae* larvae of feeding on small Chironomidae larvae. (Luferov 1961).

Species	Temperature C						
	5	10	15	20	25	30	35
Procladius nigriventris	0	24.4	86.4	152.0	52.4	31.4	0
Procladius choreus	0	24.4	84.2	177.3	53.0	36.1	0
Ablabesmyia monilis	0	35.1	96.5	271.1	247.7	37.5	0

19). At temperatures <5 and > 35 C *Procladius* larvae stop feeding. Dissections of *Procladius* larvae collected in the reservoir have proven that in winter the animals do not feed at all (Luferov 1961). Carnivorous Tanypodinae larvae have a daily rhythm, feeding more intensively in the mornings and again in the first half of the night.

One of the indices of feeding intensity is the filtering rate. The correlation between filtering rate and body weight was established with larvae feeding on suspended seston (*Glyptotendipes glaucus*, *G. imbecilis*, and *Endochironomus albipennis*) (Lukanin 1963).

The nutritive value of various kinds of food is ascertained for *Chironomus plumosus* (Sorokin and Meshkov 1959) and for *Cricotopus silvestris* larvae (Rodova and Sorokin 1965). The species of Chironomidae larvae mentioned assimilate living Protococcales very poorly. On the other hand, the index of assimilation of these algae may be high once cell walls have begun to disintegrate. Diatoms, some blue-green algae, and bacteria are well assimilated by the larvae. *Cricotopus silvestris* can also use animal food (Table 20).

Conclusions

Four main methods of feeding (filtration, scraping from the surface of substratum, predation, and mixed) have been recorded in our studies. Each of the methods may be specific either to a species, a whole family, or order. Furthermore, even very similar species may differ widely in mechanisms and character of feeding, while on the other hand, the usual accordance of methods of feeding with the structure of the mouth apparatus is not observed in some cases. Polyphagia is characteristic of most invertebrates although a few species or groups of species are stenophagous. Food spectrum and food preference in filter-feeders depend upon their filtration apparatus. In other words, only certain size categories of organisms are used as food.

Choice of food in carnivorous animals is to a considerable degree determined by accessibility of food, its mobility, the firmness of its body coverings, and the degree of dispersion and aggregation. Daily food consumption of invertebrates may change depending on concentration of food, temperature of the medium, and size of consumers. At 15–22 C and a food concentration close to optimum, mean daily rations of most invertebrates studied usually range from 25 to 100% of wet body weight. Only in pulmonary Gastropoda and silt-eating Tubificidae does it greatly exceed body weight. In rare cases, at very high concentrations of food, unusual in nature, the so-called extra feeding may take place under experimental conditions, i.e., an increase of daily ration with decreasing assimilability.

One of the indices of the effectiveness of feeding is the index of assimilation. For most invertebrates, feeding on natural foods at optimum concentrations, assimilation varies widely, rarely exceeding 50%. Percent assimilation is closely dependent upon the amount of food, and in filtrators it is usually stable at food concentrations occurring in nature. The assimilability of food, characterized by the effectiveness of digestion on one hand and the nutritive value of food on the other is 45–55% for most invertebrates studied when fed plant food, and appears

TABLE 20. Index of assimilation of various kinds of food by the *Cricotopus silvestris* larvae (Rodova and Sorokin 1965).

Food	Condition of food	Ca/W
Coelosphaerium	alive	26.6
	decayed	30.2
Nitzschia	alive	31.4
	decaying	7.1
Scenedesmus	alive	2.6
	killed	22.1
Chlorella	alive	1.0
Bacteria	"	14.7
Bosmina	"	3.9
	killed	14.1

to be considerably higher when animal food is used.

Based on the data on feeding by aquatic animals, Mordukhai-Boltovskoj (1963) offered a scheme of main trophic relations in the Volga reservoirs. Our knowledge of feeding of various invertebrates now has been augmented with new data rendering it possible to introduce some additions to the existing scheme. It is expedient to consider the open part of the reservoir and its littoral areas separately, since faunal composition and characters of food relations are different in these parts (Fig. 15, 16). In the schemes suggested only the mass species and groups of invertebrates characterised by similar types of feeding are given to avoid confusion. A specific feature of the trophic relations of the Rybinsk Reservoir is the insufficient use of phytoplankton production by heterotrophic organisms. A second feature, characteristic not only of the Rybinsk Reservoir, is the important role of Protozoa as a main source of food for numerous carnivorous Copepoda (Monakov and Sorokin 1971b). In the Rybinsk Reservoir we may outline the following ways whereby organic matter is utilized by heterotrophic organisms. The first, most simple, and apparently main way (Mordukhai-Boltovskoj, 1963) is the bacteria–detritus–herbivor ous invertebrate food chain. Bacterial food in pelagic regions is used by mass filtrators *Daphnia longispina*, *Bosmina coregoni*, benthic Ostracoda, Spheriidae, and some Chironomidae larvae; and in the coastal areas by the majority of Chironomidae, Ostracoda, Prosobranchia, and some Copepoda (Fig. 15, 16).

Besides providing a direct source of food, bacteria takes the part of an intermediary in the utilization of the dissolved organic matter by invertebrates (Sorokin and Tchardyntseva 1971).

The second way of transforming organic matter, phytoplankton–herbivorous invertebrates, is of minor importance in the Rybinsk Reservoir. In this way phytoplankton production is used in water column only by Calanoida, Rotatoria, Ostracoda, and *Dreissena* and in coastal areas by phytophyllic Chironomidae larvae, some Prosobranchia, and Cladocera (Fig. 15, 16). The products of bacterial destruction of phytoplankton are of much greater importance for feeding of herbivorous invertebrates, but via a longer food chain (Mordukhai-Boltovskoj 1963, 1965).

The character of the food web in the Rybinsk Reservoir is in many respects determined by the peculiarities of this water body (its large area and variety of habitats making coexistence of closely related species and forms possible). Coexistence is also favoured by the great variety of feeding habits and food requirements of animals, by their high trophic plasticity, and lastly by the different seasons of appearance of different invertebrates in the

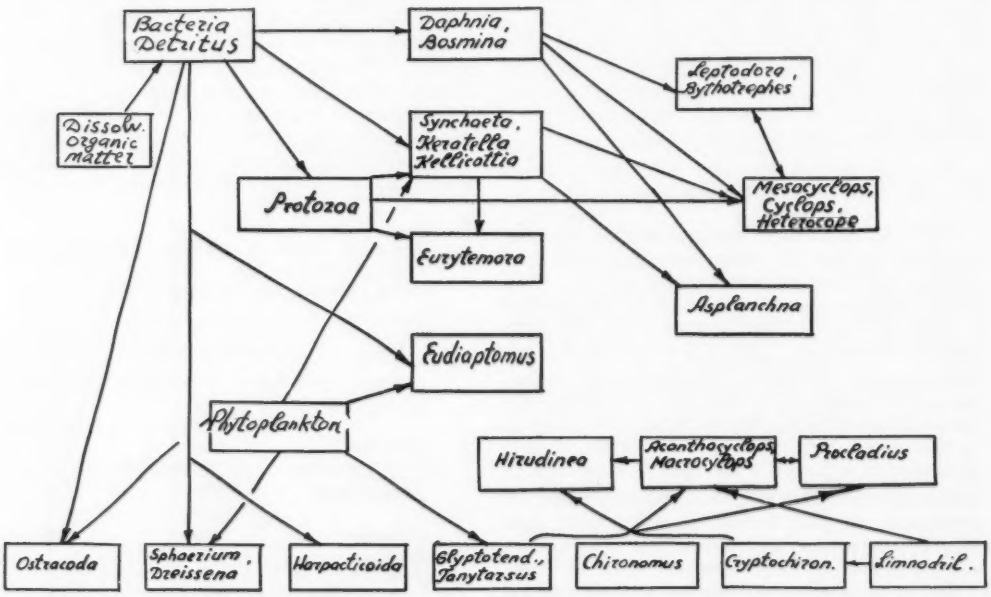

FIG. 15. Scheme of main trophic interrelations of invertebrates in the pelagic regions of the Rybinsk Reservoir.

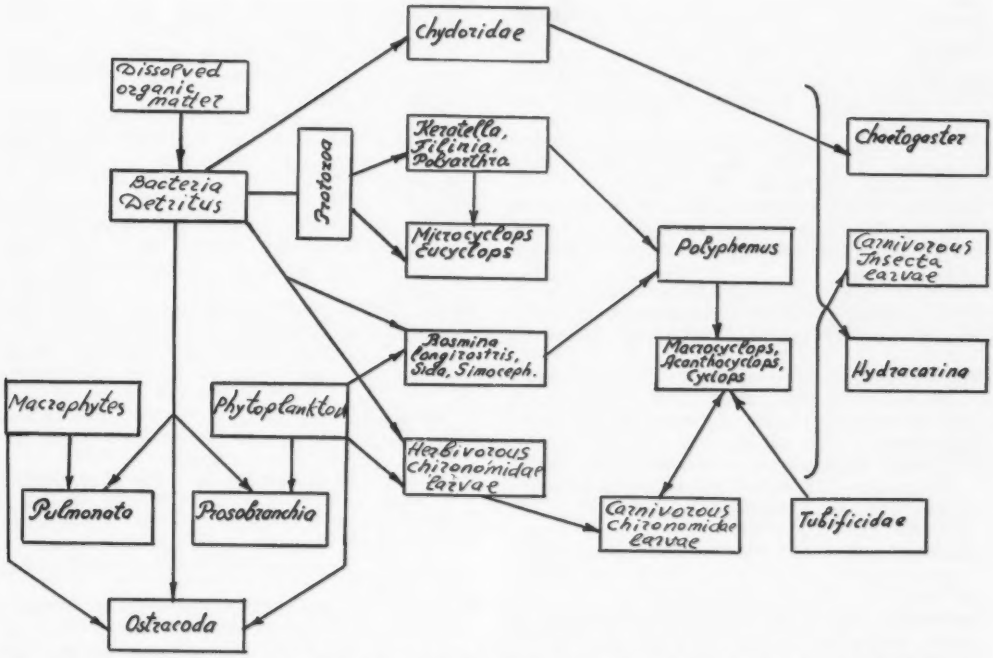

FIG. 16. Scheme of main trophic interrelations of invertebrates in the littoral zone of the Rybinsk Reservoir.

water body. In the Rybinsk Reservoir, and probably in other reservoirs of a similar type, lack of food is not likely to be felt by the inhabitants of the deep water, of the bottom and especially of the plant debris.

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Molluscan Sterols: A Review

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Many studies on mollusc sterols were made before the advent of modern techniques such as gas chromatography and mass spectrometry, and several structures ascribed to such sterols were incorrect because the preparations lacked homogeneity.

The Monoplacophora are extremely rare and the sterols have not been investigated. Cephalopods are believed to contain cholesterol but they have not been investigated in recent years. Among the Amphineura, Aplacophora have not been studied while Polyplacophora contain Δ^7-cholestenol sometimes accompanied by cholesterol and brassicasterol.

Among the least evolved pelecypods, two protobranchs studied contained cholesterol and one also had cholestenol. Septibranchia have not been investigated. A dozen species of filobranchs and eulamellibranchs have been found to have complex mixtures with the same predominant sterols. All sterols of the sea scallop have now been identified and the most recent are 22-*trans*-24-norcholesta-5,22-dien-3β-ol, and a novel C-30 compound, 29-methylisofucosterol, neither of which has been found elsewhere in nature. A primitive bivalve, the lamp shell, has an almost identical sterol composition to the modern pelecypods.

Gastropods contain predominantly cholesterol and sometimes its precursor, desmosterol.

IDLER, D. R., AND P. WISEMAN. 1972. Molluscan sterols: a review. J. Fish. Res. Bd. Canada 29: 385–398.

Les stérols des mollusques ont été l'objet de nombreuses études avant la découverte de techniques modernes telles que la chromatographie en phase gazeuse et la spectrométrie de masse, et plusieurs structures ont été attribuées incorrectement aux stérols parce que les préparations manquaient d'homogénéité.

Les monoplacophores sont extrêmement rares et leurs stérols n'ont pas été étudiés. On croit que les céphalopodes contiennent du cholestérol, mais ils n'ont pas été l'objet de recherches récentes. Parmi les amphineures, les aplacophores n'ont pas été étudiés. Les polyplacophores, par contre, contiennent du Δ^7-cholestenol accompagné parfois de cholestérol et de brassicastérol.

Parmi les pélécypodes les moins évolués, deux protobranches contiennent du cholestérol et l'un d'eux contient en plus du cholestenol. Les septibranches n'ont pas été étudiés. Une douzaine d'espèces de filibranches et d'eulamellibranches contiennent des mélanges complexes de ces stérols prédominants. Tous les stérols des pétoncles sont connus à l'heure actuelle, les plus récents étant le 22-*trans*-24 norcholesta-5-22-dien-3β-ol et un nouveau composé du C-30, le 29-méthylisofucostérol. On n'a trouvé ni l'un ni l'autre de ces derniers ailleurs dans la nature. Les térébratules, bivalves primitifs, ont une composition en stérols presque identique à celle des pélécypodes modernes.

Les gastropodes contiennent surtout du cholestérol et parfois son précurseur, le desmostérol.

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STEROLS of molluscs have been examined extensively during the past two decades by scientists of the Fisheries Research Board of Canada. Studies have dealt with identification of mollusc sterols, isolation of new marine sterols, biosynthesis, variations in sterol content, and hypocholesterolemic effects exhibited by certain sterols of bivalves. It is the purpose of this report to give a comprehensive review of these studies and the work of other scientists in the field. Results reported prior to the application of gas–liquid and thin-layer chromatography and mass spectrometry (GLC, TLC, and MS) are discussed, where possible, in relation to more recent studies.

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Several reviews of invertebrate sterols have been written (Bergmann 1949; Tarr 1958; Bergmann 1962; Brooks 1970; and Austin 1970). Austin reviewed the work of the past decade up to 1968 and re-examined some of the previous data. Since 1968, with improvements in gas–liquid chromatographic systems and the use of mass spectrometry, there have been several additions to the knowledge of mollusc sterols.

This review considers the following eight topics and concludes with a summary; a list of mollusc sterols, systematic nomenclature, and empirical formulae precedes the references:

Sterols of Amphineura
Sterols of Pelecypoda
  Analyses without the use of GLC
  GLC analyses
  $\Delta^{5,7}$ sterols
Sterols of Gastropoda
  Analyses without the use of GLC
  GLC analyses
  $\Delta^{5,7}$ sterols
Sterols of Cephalopoda
Qualitative variations in mollusc sterols
Quantitative variations in mollusc sterols
Biosynthesis in mollusc sterols
  Pelecypoda
  Gastropoda
  Cephalopoda
Hypocholesterolemic factors in mollusc sterols
Summary

Sterols of Amphineura

All analyses are of chitons belonging to the order Polyplacophora and all contain Δ^7-cholestenol (lathosterol). This sterol has been isolated from chitons common to Japanese waters, *Liolophura japonica* (Toyama and Tanaka 1953; Takagi and Toyama 1958), and *Acanthochiton rubroilneatus* (Takagi and Toyama 1958), as well as a chiton native to Bermuda waters, *Chiton tuberculatus* (Kind and Meigs 1955). In the latter study, the authors indicated that the isolated sterol was pure and that there was virtually no contamination with cholesterol or C_{28} and C_{29}-sterols. This assumption was made by comparing the melting point and optical rotation of the isolated sterol with reference Δ^7-cholestenol. In a recent study using GLC and MS, eight sterols were found in *Chaetopleura apiculata* (Idler and Wiseman 1971b) and Δ^7-cholestenol was 89.7% of the total sterols; cholesterol, 2.8%; brassicasterol, 1.2%; and 22:23-dihydrobrassicasterol or its C_{24}-isomer, campesterol, 2.0%. Another chiton (species unidentified) contained three sterols; Δ^7-cholestenol, 75%; cholesterol, 24.7%; and brassicasterol, 0.3%. These findings indicate that chitons are similar to the majority of marine invertebrates in that they contain mixtures of many sterols. They remain unique within the phylum Mollusca, however, in having Δ^7-cholestenol as the principal sterol. Chitons feed on seaweeds and plankton, which they scrape from rocks, and most of which contain principally Δ^5-sterols (Bergmann 1962; Austin 1970). Thus, chitons must be capable of either converting ingested sterols into Δ^7-cholestenol or synthesizing sterols from basic precursors. It is of interest that both Δ^7-sterols and Δ^5-sterols are present in the same animal. Fagerlund and Idler (1960) showed that starfish can transform the Δ^5-sterols, which they ingest, into the Δ^7-type; it is possible that similar bioconversions take place in chitons.

Sterols of Pelecypoda

The pelecypods (bivalves) contain complex mixtures of Δ^5-sterols varying in side-chain length, and nuclear and side-chain unsaturation. Early literature on mollusc sterols contains many common names given to supposedly new sterols. Elucidation of sterol structure is lacking in most cases, mainly because efficient techniques for separating and identifying sterols had not been developed. Minor sterols remained unnoticed and mixtures inseparable. Although many of the early identifications of pelecypod sterols are now known to be erroneous, they will be included in this study as a historical review. The use of GLC proved that mollusc sterols are indeed complex mixtures and as many as 13 sterols have been found in a single species. Pure sterols can be isolated by preparative GLC and TLC.

Analyses Without the Use of GLC

From the earliest investigations of pelecypod sterols, cholesterol has been identified as a major component. However, as early as the 1930's it was recognized that other sterols were present in bivalves. In 1943, a sterol named "corbisterol" was separated from the fat of the clam, *Corbicula leana* (Matsumoto and Toyama 1943). Its empirical formula was suggested as either $C_{29}H_{44}O$ or $C_{28}H_{42}O$ (a tetra-unsaturated sterol) on the basis of iodine number and saponification equivalent. Its identity as a mixture of 22:23-dihydroergosterol (a 5, 7-diunsaturated sterol) with its C_{24}-methyl epimer (7-dehydrocampesterol) was later suggested (Bergmann and Ottke 1949). In the 1950s, Japanese investigators examined *Corbicula* again and isolated four sterols; corbisterol, poriferasterol, clionasterol (γ-sitosterol), and cholesterol (Toyama and Tanaka 1954). Corbisterol, now identified with 7-dehydrostigmasterol (a C_{29}-sterol), was isolated from the fat of *Cristaria spatiosa* (Matsumoto and Tamura 1955b), *Venerupis semidecussata* (Matsumoto and Tamura 1956), *C. leana*, and *C. japonica* (Tamura et al. 1956).

"Brassicasterol" was one of the earliest mollusc sterols to be isolated as a pure component and to retain its identity today. Matsumoto and Toyama identified the sterol in *C. leana* (1944a) and the oyster *Ostrea gigas* (1944b). Bergmann and Low (1947) suggested that a sterol isolated earlier by them from the oyster *Ostrea virginica* was brassicasterol rather than stigmasterol, and that "shakosterol" isolated from *Tridacna gigas* (Tsujimoto and Koyanagi 1935) was also identical with brassicasterol. The mussel *Modiolus demissus* contained significant quantities of brassicasterol as well as cholesterol (Bergmann and Ottke 1949). Brassicasterol was isolated from this species by formation of the difficultly soluble acetate tetrabromide and obtained by fractionation of the acetate bromide mixture.

"Conchasterol" was isolated from nine pelecypods and thought to be an isomer of cholesterol (Tsujimoto and Koyanagi 1934a, 1934c). "Meretristerol," a $C_{28}H_{46}O$ sterol, was isolated and believed to be identical with conchasterol (Toyama and Yajima 1943). However, identifications of both sterols were indefinite, and Bergmann (1949) suggested that these two sterols were mixtures of brassicasterol and "chalinasterol" (chalinasterol was later shown to be a mixture, see below). It is obvious why confusion existed, with such a multitude of trivial names for poorly characterized sterols!

Kind and Goldberg (1953) studied the sterols of the bivalve *Anomia simplex* and found only a trace of cholesterol. They suggested the main sterols were a mixture of C_{28}-sterols, chalinasterol, and brassicasterol. Toyama and Takagi (1954) mentioned that *Spisula sachalinensis* probably contained β-sitosterol and a sterol called "cryonasterol." Ultraviolet spectra of the crude sterol indicated the presence of 5.6% provitamin D. Takagi et al. (1956) identified 7-dehydrostigmasterol, poriferasterol, and clionasterol in *Cynthia roretzi*, and clionasterol along with a $\Delta^{5,7}$-sterol in *Pinna pectinata japonica*. *Mytilus edulis* apparently contained a mixture of four sterols: poriferasterol, clionasterol, β-sitosterol, and cholesterol (Toyama and Tanaka 1956). Ashikaga (1957) isolated a supposedly new sterol, "pincsterol" (C_{24}-epimer of brassicasterol?) from the pearl oyster *Pinctada martensi*.

The case of "chalinasterol" and "ostreasterol" is an example of an erroneous identification of a poorly characterized, heterogeneous sterol mixture. In 1934, Bergmann (1934a, 1934b) isolated a new sterol from three pelecypods; oyster *Crassostrea virginica*, quahog *Venus mercenaria*, mussel *Modiola* sp., and two gastropods *Fulgur* sp. He called the sterol "ostreasterol" and thought it was isomeric with stigmasterol. Later he suggested it was identical with the "chalinasterol" which had been isolated by Japanese workers; the sterol was assigned the structure $\Delta^{5,22(23)}$-campestadiene-3β-ol (Bergmann and Low 1947). The evidence seemed to be based solely on a resemblance of physical properties. The isolation and identification of 24-methylenecholesterol in the mid-1950s cleared the existing confusion and proved both sterols to be heterogeneous (Idler and Fagerlund 1957b). Infrared spectra showed that "chalinasterol" and "ostreasterol" contained approximately 30–50% of a sterol with a terminal methylene group. 24-Methylenecholesterol was first isolated, from the sterols of the oyster *Ostrea gigas* and clam *Saxidomus giganteus*, by chromatography of the azoyl esters on silicic acid (Idler and Fagerlund 1955), and subsequently from three other pelecypods, scallop *Pecten caurinus*, cockle *Cardium corbis*, and mussel *Modiolus demissus* (Fagerlund and Idler 1956). Oyster and clam sterols contained 36 and 53% 24-methylenecholesterol, respectively. The sterol was also synthesized from 24-ketocholesterol and its identity with the natural product was established (Idler and Fagerlund 1957a). There is no doubt that 24-methylenecholesterol was a component of many of the earlier pelecypod sterols. Kita and Toyama (1960) isolated 24-methylenecholesterol and clionasterol from *Pecten yessoensis*, and mass spectral studies were used to identify 24-methylenecholesterol in the oyster *Ostrea gryphea* (Salaque et al. 1966).

Takanashi and Mitsuhashi (1968) examined sterols of the mussel *Mytilus edulis* and found principally β-sitosterol. This is of interest since this sterol is not a principal component of any mollusc sterol mixture analysed by GLC. (Nine sterols were present in *Mytilus edulis* examined by Idler and Wiseman (1971b) with GLC, and β-sitosterol was only 1.2% of the total sterol, Table 1).

GLC Analyses

Wainai et al. (1964) analyzed free and hydrogenated sterols of the scallop *Placopecten magellanicus* by GLC on 5% SE-30, and sterol methyl ethers on 5% diethylene glycol succinate (DEGS). Total scallop sterols resolved into eight components; hydrogenated sterols into five; and sterol methyl ethers into ten. The hydrogenated sample contained: C_{26}-sterol (6.1%), C_{27}-sterols (42.4%), C_{28}-sterols (41.9%), C_{29}-sterols (9.6%), and C_{30}-sterol ($< 1\%$). Since scallop sterol methyl ethers were not completely resolved by GLC on 5% DEGS, it was desirable to search for a more efficient system. This was obtained by mixing two liquid phases, 3% XE-60 and 3% NGS (1:1) coated on 100/120 mesh Gas Chrom Q, and using a longer (14 ft long, 5/16 inches ID), glass, coiled column (Idler and Wiseman 1971a). Scallop sterols were re-examined with this system as methyl ether derivatives and 11 peak areas were

TABLE 1. Percentage composition of pelecypod sterols determined by GLC.

Sterols	*Solemya velum*[a]	*Nucula* sp.[a]	*Mytilus edulis*[a]	*Crassostrea virginica*[a]	*Arctica islandica*[a]	*Spisula solidissima*[a]	*Mya arenaria*[a]	*Terebratalia transversa*[a] (a brachiopod)	Clam[b]	Scallop[b]	Oyster[b]	*Tapes japonica*[c]
22-*trans*-24-norcholesta-5,22-dien-3β-ol	–	0.3	5.9	4.6	5.5	2.7	2.4	3.8	4.8(?)	4.4(?)	5.6(?)	–
22-*cis*-cholesta-5,22-dien-3β-ol	–	0.3	0.9	1.1	4.3	1.9	0.5	0.6	7.9	13.4	2.9	7.2
22-*trans*-cholesta-5,22-dien-3β-ol	–	8.6	8.6	7.5	5.5	2.9	4.0	4.6				
cholesterol	100	63.2[d]	58.7	34.1	44.5	68.0	59.0	32.2	36.7	25.7	41.4	35.4
brassicasterol	–	10.7	9.6	28.1	7.0	8.5	12.3	16.7	14.1	14.1	16.0	15.7
desmosterol	–	–	8.1	–	1.7	–	2.0	–	–	–	–	–
7-dehydrocholesterol	trace	–	–	0.4	–	–	–	0.6	–	–	–	–
22:23-dihydrobrassicasterol or campesterol	–	5.4	0.6	1.7	4.2	3.4	3.3	3.9	–	–	–	–
poriferasterol	–	5.9	6.4	1.5	–	–	–	–	–	–	–	–
24-methylenecholesterol	–		–	8.1	21.6	5.4	10.8	20.4	20.2	19.5	25.9	25.8
β-sitosterol	–	5.6	1.2	3.7	3.3	4.8	4.1	–	–	–	–	–
fucosterol	–	–	–	0.1	–	–	–	2.8	–	–	–	–
28-isofucosterol	–	–	–	8.3	2.4	2.4	1.6	3.3	–	–	–	14.2
unidentified sterols	–	–	–	0.8	–	trace	–	10.5	16.2	8.5	8.2	–

[a]Idler and Wiseman 1971b.
[b]Kritchevsky et al. 1967.
[c]Yasuda 1970.
[d]Mixture of cholestanol and cholesterol.

obtained (Fig. 1). Nine sterols, including two new marine sterols, were isolated by preparative GLC of the scallop sterol trimethylsilyl ethers on 3% OV-17 (Idler and Wiseman 1971a). The structure of one new marine sterol, 22-*trans*-24-norcholesta-5,22-dien-3β-ol (Fig. 2) was elucidated by MS, nuclear magnetic resonance spectrometry and IR spectroscopy (Idler et al. 1970c). This sterol was 6.3% of the mixture. It is the first isolation of a naturally occurring C_{26}-sterol and the authors have detected it in many other marine species. The second new marine sterol was 22-*cis*-cholesta-5,22-dien-3β-ol (Fig. 2) (Idler and Wiseman 1971a). Tamura et al. (1964b) had earlier isolated 22-dehydrocholesterol from scallop sterols but had not detected the 22-*cis* and 22-*trans* isomers. Both isomers were completely resolved on the new mixed phase GLC system. Other scallop sterols isolated and identified by GLC and MS were 22-*trans*-cholesta-5,22-dien-3β-ol, cholesterol, 24-methylenecholesterol, brassicasterol, β-sitosterol, 28-isofucosterol, and a $C_{30}H_{50}O$ sterol (Idler and Wiseman 1971a).

Sterol compositions of seven pelecypods (Table 1) were determined by GLC of the sterol methyl ethers and confirmation of the identity of the principal components was obtained by the presence of molecular ions and characteristic fragmentation patterns in the mass spectra (Idler and Wiseman 1971b). Two species showed sterol compositions unique among the Pelecypoda; the sterol of *Solemya velum* was almost pure cholesterol, and a mass spectrum of the sterols of *Nucula* sp. revealed a larger amount

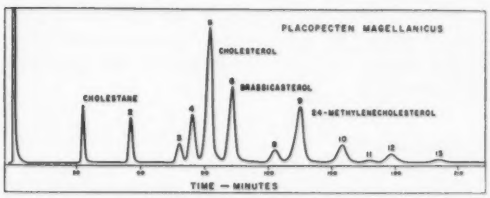
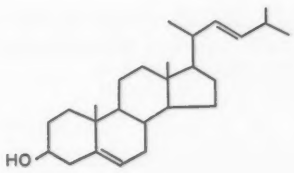
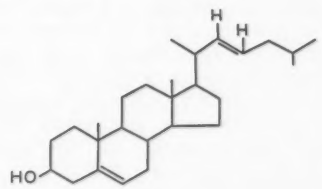


FIG. 1. GLC separation of methyl ether derivatives of scallop (*Placopecten magellanicus*) sterols on 3% XE-60 and 3% NGS (1:1). The percentage composition and retention time of each sterol relative to cholestane (R_c) is given below. Retention times of reference sterols are given in brackets (Idler and Wiseman 1971a).
(1) 6.3% 22-*trans*-24-norcholesta-5,22-dien-3β-ol, 1.68; (2) 3.7% 22-*cis*-cholesta-5,22-dien-3β-ol, 2.37 (2.42); (3) 10.9% 22-*trans*-cholesta-5,22-dien-3β-ol, 2.56(2.56); (4) 31.8% cholesterol, 2.78 (2.78); (5) 17.7% brassicasterol, 3.12 (3.15); (6) 3.0% 22:23-dihydrobrassicasterol, 3.77 (3.76) or campesterol (3.81); (7) 17.4% 24-methylenecholesterol, 4.10 (4.12); (8) 5.4% β-sitosterol, 4.73 (4.76); (9) 0.4% fucosterol, 5.15 (5.24); (10) 2.7% 28-isofucosterol, 5.45 (5.57); (11) 0.7% $C_{30}H_{50}O$ sterol, 6.15.



22-TRANS-24-NORCHOLESTA-5,22-DIEN-3β-OL



22-CIS-CHOLESTA-5,22-DIEN-3β-OL

FIG. 2. Two recently identified sterols of scallop (*Placopecten magellanicus*) (Idler et al. 1970c; Idler and Wiseman 1971a).

of cholestanol than cholesterol (36% to 26% relative intensity of the molecular ion). Cholestanol had not previously been detected as a major sterol in molluscs. Unfortunately, the mixed-phase GLC system does not separate cholesterol from its saturated analogue; this separation can be obtained with TLC on silver-nitrate-impregnated silicic acid.

Kritchevsky et al. (1967) studied sterols of oyster, clam, and scallop with GLC on 4.1% SE-52 (Table 1). The percentage composition of scallop sterols is similar to the composition of *Placopecten magellanicus* (Fig. 1). Yasuda studied sterols of the Japanese littleneck clam *Tapes japonica*. In 1966, he identified the principal sterol as 7-dehydrostigmasterol. The presence of another sterol was indicated by IR spectra and TLC. On re-examination of this species by GLC of the sterol methyl ethers on 5% DEGS, at least 10 peak areas were detected (Yasuda 1970). The sterols were not completely resolved by GLC, but the principal components were isolated by column chromatography on silver-nitrate-impregnated silicic acid (Table 1).

Sterols of a brachiopod, *Terebratalia transversa* (the red lamp shell), are of particular interest as they closely resemble the sterols of certain pelecypods (scallop, oyster). This species showed a mixture of 15 sterols on GLC analysis; the major components were cholesterol (32.2%), 24-methylenecholesterol (20.4%), and brassicasterol (16.7%) (Idler and Wiseman 1971b). Brachiopods (phylum, Brachiopoda

or Molluscoidae) superficially resemble bivalve molluscs, but the animals contained within the shells are more closely related to the Bryozoa.

$\Delta^{5,7}$-STEROLS

Provitamin D sterols vary considerably in pelecypods. They are abundant in some species, absent in others, but generally present as minor components in mixtures of Δ^5-sterols. The presence of corbisterol (7-dehydrostigmasterol) in a number of species was discussed earlier. Petering and Waddell (1951) isolated a new provitamin D from the crude sterols of *Modiolus demissus*. Its molecular weight was approximately 410, indicating a C_{29}-sterol. Toyama and Takagi (1954) found a provitamin D in *Spisula sachalinensis* (5.6% of sterols) and Tanaka and Toyama (1960) found $\Delta^{5,7}$-sterols in *Mya arenaria* (12.6%) and *Pinna pectinata* (15.8%). Fagerlund and Idler (1956) found no significant quantities of $\Delta^{5,7}$-sterols in scallop, cockle, and mussel. In monthly samples of *Placopecten magellanicus*, a range of 0.41–3.0% $\Delta^{5,7}$-sterols was found; and in a recent examination of seven pelecypods (Idler and Wiseman 1971b), only trace amounts of $\Delta^{5,7}$-sterols were present (Table 1). The presence of $\Delta^{5,7}$-sterols was indicated in *Tapes japonica* (Yasuda 1970).

Sterols of Gastropoda

Analyses Without the Use of GLC

Early studies of gastropod sterols indicated cholesterol as the principal sterol of all species with the exception of *Buccinum undatum*, which contained principally 7-dehydrocholesterol (Boch and Wetter 1938). In most species only one or two sterols were identified. Tsujimoto and Koyanagi (1934b) found cholesterol and small amounts of the pelecypod sterol "conchasterol" in ear and top shells, and Bergmann (1934a) isolated cholesterol and "ostreasterol" from *Fulgur carica* and *Fulgur canaliculata*. Kind et al. (1948) identified cholesterol in the "basket shell" *Nassa obsoleta* and the "bleeding tooth" *Nerita peleronta*, and suggested that an unidentified sterol in the former species was clionasterol. The periwinkle *Littorina littorea* contained cholesterol (85%) and clionasterol (Kind and Herman 1948), with the presence of 7-dehydroclionasterol suggested. In the third study of a series, Kind and Goldberg (1953) found that the sterols of the oyster drill *Urosalpinx cinereus* consisted of 90% cholesterol and 10% $\Delta^{5,7}$-sterol. Four sterols, cholesterol, brassicasterol, "polypherasterol", and γ-sitosterol were isolated from a snail, *Viviparus japonicus*. $\Delta^{5,7}$-Sterols were detected by UV spectrophotometry (Matsumoto and Tamura 1955a). Toyama et al. (1955) found cholesterol in *Turbo cornutus*, *Lunella coronata coreensis*, and *Monodonta labio*, and Toyama and Tanaka (1956) found the same sterol in *Buccinum perryi* and *Tegula argyrostoma sublaevis*. Tanaka and Toyama identified a tetra-unsaturated sterol, $\Delta^{5,7,22,25}$-cholestatetraenol (1957), as well as cholesterol and clionasterol (1958) in *Tonna luteostoma*, and cholesterol and 2.2% $\Delta^{5,7}$-sterols in *Littorina brevicula* (1960).

GLC Analyses

Voogt (1969) studied the sterols of two snails *Viviparus fasciatus* and *Littorina littorea* by GLC in four different systems, and Idler and Wiseman (1971b) examined the sterols of 10 gastropods by GLC of the sterol methyl ether derivatives on the mixed-phase column of 3% XE-60 and 3% DGS, and by MS of the free sterols. Sterol compositions of the Gastropoda examined in these two studies are given in Table 2. Mitsuhashi et al. (1970) found cholesterol and a small amount of β-sitosterol in a snail, *Achatina fulica*, on TLC and GLC analyses.

Baalerud (1950) examined sterols of two pteropods, *Clione limacina* and *Limacina helicina*, without GLC and isolated "pteropodasterol". The pteropod *Spiratella helicina* (*Limacina helicina*) showed an interesting mixture (Table 2) of 11 sterols on GLC analysis (Idler and Wiseman 1971b). Cholesterol comprised only 45.6% of the total sterol, and other major components were β-sitosterol (12.0%), desmosterol (9.8%), and 24-methylenecholesterol (8.1%). It is probable that "pteropodasterol" was actually a complex mixture of sterols. In all analyses of gastropod sterols by GLC, sterol mixtures have been found and in all cases cholesterol is the principal component.

$\Delta^{5,7}$-STEROLS

In one of the earliest studies of mollusc sterols, Boch and Wetter (1938) found that sterols of the whelk *Buccinum undatum* contained 78% 7-dehydrocholesterol. In later studies, $\Delta^{5,7}$-sterols were found in some species, but usually in amounts less than 10% of the total sterol. The report of a $\Delta^{5,7}$-sterol as the principal component of gastropod sterols has not been confirmed. Occurrence of a possible tetra-unsaturated sterol in *Tonna luteostoma* has been mentioned. Tanaka and Toyama (1960) found 2.2% $\Delta^{5,7}$-sterol in *Littorina brevicula* and 11% in *Dendrodoris rubra* var. *nigromaculata*. *Viviparus japonicus* and *Littorina littorea* were void of $\Delta^{5,7}$-sterols (Voogt 1969), and Idler and Wiseman found only trace amounts ($< 1\%$) in three species (Table 2).

TABLE 2. Percentage composition of gastropod sterols determined by GLC.

Sterols	*Amacea testudinalis alvea*	*Lunatia groenlandica*[a]	*Crepidula fornicata*[a]	*Littorina littorea*[a]	*Urosalpinx cinerea*[a]	*Buccinum undatum*[a]	*Neptunea decemcostrata*[a]	*Busycon canaliculata*[a]	*Spiratella helicina*[a]	*Melampus lineatus*[a]	*Viviparus fasciatus*[b]	*Littorina littorea*[b]
22-*trans*-24-norcholestal-5,22-dien-3β-ol	0.1	0.2	2.8	0.9	trace	1.5	0.6	0.2	3.7	1.0	trace(?)	0.3(?)
22-*cis*-cholesta-5,22-dien-3β-ol	–	trace	0.4	trace	–	trace	0.3	0.1	1.0	trace	–	–
22-*trans*-cholesta-5,22-dien-3β-ol	0.3	0.4	2.8	1.7	0.5	1.1	1.3	0.5	4.6	1.9	<1	–
cholesterol	97.0	98.0	74.0	56.0	98.5	94.4	93.5	85.5	45.6	85.1	70	64
brassicasterol	trace	0.4	4.4	4.3	trace	0.3	0.7	0.8	4.2	3.7	5	5
desmosterol	2.0	0.1	–	29.5	–	2.0	–	–	9.8	–	–	20
7-dehydrocholesterol	–	–	–	–	–	–	–	trace	–	0.9	–	–
22:23-dihydrobrassicasterol or campesterol	trace	0.4	2.8	1.9	1.0	trace	1.1	8.1	3.5	2.2	<6	–
poriferasterol	–	–	–	–	–	–	–	0.4	–	–	–	–
24-methylenecholesterol	0.6	–	3.9[c]	1.8	–	0.7	2.5	–	8.1	1.9	trace	4
β-sitosterol	–	0.4	5.9	–	–	–	trace	3.7	12.0	3.1	10	3
fucosterol	–	–	–	0.6	–	–	trace	–	1.8	–	1	2
28-isofucosterol	trace	0.1	3.0	1.6	–	–	–	0.7	5.7	–	–	–
unidentified sterols	trace	–	–	1.4	–	–	–	3.7	–	–	–	–

[a]Idler and Wiseman 1971b.
[b]Voogt 1969: Stigmasterol (4%) in *Viviparus*; 4% chondrillasterol (?) in *Littorina littorea*; Δ^{22}-cholestenol (?) in both species.
[c]A small amount of ergosterol in this peak.

Sterols of Cephalopoda

Cephalopods are the most highly developed molluscs. Cholesterol is the only sterol in the cuttlefish of Atlantic waters *Sepia officinalis* (Deffner 1943; Zandee 1967) and the japanese cuttlefish (Marumo et al. 1955). This indicates an evolutionary trend within molluscs towards a single sterol, cholesterol. It would be interesting, however, to re-examine the cephalopods by GLC as minor sterols could have gone undetected.

Qualitative Variations in Mollusc Sterols

Certain molluscs of the same species have different sterol compositions when taken from distant localities, whereas other species are surprisingly similar. In one case, β-sitosterol was identified as the main sterol of *Mytilus edulis* (Takanashi and Mitsuhashi 1968), and in another analysis, this species contained a complex mixture of nine sterols with cholesterol 58.7% of the total sterols (Table 1). Similarities in sterol compositions were noted in *Littorina littorea* (Table 2). It is not known what effects differences in locality of native waters and dietary sterols have on the sterol composition of a species. Tamura et al. (1964a) suggested that sterols of the oyster *Crassostrea virginica* were partly of exogenous origin and partly endogenous (see section on Biosynthesis of mollusc sterols). Seasonal changes in sterol composition do not appear to be extreme, but studies have been limited. Monthly samples of the scallop *Placopecten magellanicus* were analyzed by GLC (Idler et al. 1964); the cholesterol content per unit weight of flesh increased in March and was offset by changes in the 24-methylenecholesterol content. The percentage of brassicasterol and 22-dehydrocholesterol remained fairly constant.

Quantitative Variation in Mollusc Lipids

Considerable variations in total fat, unsaponifiable matter, and sterols have been obtained for various species of molluscs (Table 3). These variations may be explained by a combination of factors including species, geological location, season of collection, portion of body analyzed, and methods of analyses. Voogt (1968a) showed that extracting unsaponifiable lipids with diethyl ether gave a yield six times greater than was obtained with petroleum ether. This author also noted that freshwater gastropods have lower lipid contents than marine gastropods.

Ashikaga (1950) studied seasonal variations in the meat of the pearl oyster *Pinctada martensi*. Total fat accumulated gradually during the winter and was at a maximum level in June (8.04% on dry weight basis). There was a sudden decrease in July with delivery of the spermatozoa and eggs (5.06%). Total unsaponifiable matter was highest in March (2.03%) and lowest in July (1.32%). The sterol content remained constant throughout the year (0.6–0.7%) except in July when it dropped to 0.45%.

Idler et al. (1964) studied seasonal variation of sterols in a bivalve, the scallop *Placopecten magellanicus*. Scallops were collected monthly for a 1-year period from the same sea beds. Males and females were analyzed separately but showed no differences related to the sex of the animal. Total fat content dropped sharply in August but was also low from January to March (contrary to Ashikaga's findings for the late winter). In both studies, the maximum level of total fat was in June. It is of interest in scallops, when the fat level was low in August, there was a corresponding low percentage of unsaponifiable material and sterols, whereas the low fat content in late winter samples was offset by high unsaponifiable and sterol values. In relation to the weight of the meat, the content of unsaponifiable matter and sterols remained fairly constant throughout the year except in August, when they dropped sharply. The authors speculated that the low sterol values in August indicate an active transfer of fatty material from muscle tissue to gonads.

Biosynthesis of Mollusc Sterols

The origin of complex mixtures of sterols in molluscs is of great interest. Related to this question is the function of the individual sterols, either structural or metabolic or both. Studies on the biosynthesis of sterols in the Pelecypoda, Gastropoda, and Cephalopoda will be considered independently.

Pelecypoda

Sterol biosynthesis in marine invertebrates was first reported in the mussel *Mytilus californianus* and clam *Saxidomus giganteus*, injected with 2-C^{14}-acetate (Fagerlund and Idler 1960). Clams were also capable of converting C^{14}-labelled squalene into sterols, suggesting a role for squalene as a precursor in sterol biosynthesis. Through chromatography of the azoyl esters, the major portion of the radioactivity (80%) was found in the zone containing mono-unsaturated Δ^5-sterols. An insignificant amount of radioactivity was found in the zone containing 24-methylenecholesterol, although this sterol was the major component in the sterol mixture. When cholesterol-26-C^{14} was injected into clams, it was shown that the sterol side chain can undergo unsaturation at C-22 and C-25, and this is discussed in relation to the hypothesis advanced by Tschesche and Korte (1955) as to the origin of C_{28} and C_{29}-sterols in

plants. Briefly, this theory states that the activation of C-24, by a system of C-22 and C-25 double bonds, would facilitate the addition of one or two carbon-units at C-24. An intermediate with three double bonds would first be formed and by hydrogenation would give rise to C_{28}- and C_{29}-sterols. Thus, 24-methylenecholesterol in clams could be derived from a precursor with a cholesterol-type side chain. If 24-methylenecholesterol has a structural function in clams, the turnover rate of this sterol would be very slow, thus explaining the absence of radioactivity in the 24-methylenecholesterol zone when 2-C^{14}-acetate was injected. The Tschesche-Korte hypothesis would also explain the presence of such a variety

TABLE 3. Total fat, unsaponifiable matter, and sterols calculated as weight % of the fresh weight of meat.

Species	Fats	Unsap. matter	Sterols	
	% of meat	% of meat	% meat	% unsap.
Class Amphineura				
Chiton tuberculatus (1)[a]	1.1			30.0
Loliophura japonica (2)	1.05			44.8
Class Pelecypoda				
Pinna pectinata (3)				67.04
Mya arenaria (3)				56.65
Mytilus edulis (4)				48.3
Modiola adriatica (5)			avg. 0.12	
Ostrea taurica (5)			avg. 0.07	
Pecten ponticus (5)			avg. 0.03	
Mytilus galloprovincialis (5)			avg. 0.06	
Mercenaria mercenaria (6)	2.0		avg. 0.08	
Aquapecten grandis (6)	1.6		avg. 0.06	
Crassostrea virginica (6) (eastern)	2.0		avg. 0.06	
Crassostrea virginica (6) (southern)	2.4		avg. 0.04	
Crassostrea virginica (7)	2.0	0.41	0.14	
Mya arenaria (8)	0.64	0.15	0.07	
Arctica islandica (8)	0.58		0.04	
Spisula solidissima (8)	0.63	0.08	0.06	
Class Gastropoda				
Dendrodoris rubra var. *nigromaculata* (3)				26.08
Littorina breviculata (3)				46.23
Arion rufus L. (9)	0.65	0.24	0.12	
Patella coerulea (10)		0.21	0.07	
Monodonta turbinata (10)		0.45	0.17	
Urosalpinx cinereus (11)	0.64	0.08	0.04	
Viviparus fasciatus (12)	0.72	0.10	0.07	
Littorina littorea (12)	5.03	0.70	0.04	
Buccinum undatum (13)	0.62	0.50	0.06	
Buccinum undatum (8)	0.83	0.21	0.11	
Busycon (8)	0.57	0.11	0.08	
Crepidula fornicata (8)	0.74	0.46	0.05	
Spiratella helicina (8)	1.55	0.24	0.08	
Planorbarius corneus (14)		0.2	0.01	
Limnaea stagnalis (14)		0.3	0.01	
Limnaea peregra (14)		0.19	0.03	

[a]References, (1) Kind and Meigs 1955; (2) Toyama and Tanaka 1953; (3) Tanaka and Toyama 1960; (4) Takanashi and Mitsuhashi 1968; (5) Vinogradova 1957; (6) Thompson 1964; (7) Tamura et al. 1964a; (8) D. R. Idler unpublished data; (9) Voogt 1967a; (10) Voogt 1968b; (11) Kind and Goldberg 1953; (12) Voogt 1969; (13) Voogt 1967b; (14) Voogt 1968a.

of $\Delta^{5,22}$-sterols in molluscs. In a third study of this series, clams were able to convert injected cholesterol-4-C^{14} into radioactive 24-methylenecholesterol in vivo (Fagerlund and Idler 1961b). The highest rate of conversion was observed when the radioactive sterol was injected into the digestive gland.

The effects of dietary sterols on the sterols of oyster, *Crassostrea virginica*, was studied by Tamura et al. (1964a). One group of oysters was fed a sterol-free diet and another group a diet supplemented with β-sitosterol (a C_{29}-sterol), for a total period of 32 weeks. A depletion of sterols in the flesh of oysters on the sterol-free diet suggested that oyster sterols are at least in part of exogenous origin. The relative cholesterol content increased in the flesh of oysters on a sterol-free diet, but remained fairly constant for the experimental group. In both groups the amount of 24-methylenecholesterol remained constant relative to other sterols. The oysters were apparently capable of synthesizing cholesterol and converting it to 24-methylenecholesterol, as demonstrated in clams earlier. β-sitosterol added to the diet of the experimental group was digested by the oyster and accumulated in the endogenous sterols. A C_{29}-sterol (28-isofucosterol, see Table 1) decreased in both groups, indicating that it may be an essential sterol.

Salaque et al. (1966) reported that the oyster *Ostrea grypha* does not incorporate 2-C^{14}-mevalonate or C^{14}-CH_3-L-methionine into squalene or sterols. These negative results could be explained by the short duration of the experiments (66 and 70 hr, respectively), particularly if the biosynthesis of sterols in oyster is extremely slow.

Gastropoda

Boch and Wetter (1938) found ergosterol in two snails, *Arion empiricorum* (25%) and *Helix pompalia*, and concluded that these animals are capable of synthesizing this sterol because it was lacking in their diets. These snails are phytophagous representatives of the subclass Pulmonata, order Stylommatophora. Addink and Ververgaert (1963) proved that the snail *Helix pompalia* L. could synthesize cholesterol from sodium-1-C^{14}-acetate, and Voogt (1967a) found similar results for *Arion rufus* L. Several other snails are capable of sterol biosynthesis; *Limnaea stagnalis*, *Limnaea peregra* f. *ovata*, and *Planorbarius corneus* of the subclass Pulmonata, order Basommatophora (Voogt 1968a); *Patella coerulea* L. and *Monodonta turbinata* of the subclass Prosobranchia, order Archeogastropoda (Voogt 1968b); and *Viviparus fasciatus* and *Littorina littorea* of the subclass Prosobranchia, order Mesogastropoda (Voogt 1969). All of these snails are phytophagous and all contain cholesterol as the principal sterol. Thus, cholesterol must be either synthesized from metabolic products or be modified from ingested sterols. Evidence points to the former speculation. The land slug *Ariolimax californicus* is also capable of cholesterol biosynthesis (Gottfried and Dorfman 1969).

In contrast to snails, the whelk *Buccinum undatum*, a neogastropod and carnivorous animal, is incapable of synthesizing 3β-sterols (Voogt 1967b). Oyster drill (*Urosalpinx cinerea*) sterols are mainly cholesterol (98.5%) with 1% C_{28}-sterol (see Table 3). Since the oysters on which this species feeds contain complex sterol mixtures, including C_{28}- and C_{29}-sterols, the oyster drill would seem to be either capable of utilizing the ingested sterols or it does not absorb them.

Cephalopoda

A carnivorous mollusc, the cuttlefish *Sepia officinalis*, did not incorporate sodium acetate-1-^{14}C into sterols during a 24-hr incubation period (Zandee 1967).

Hypocholesterolemic Factors in Mollusc Sterols

Reiner et al. (1960) found that the sterols of the butter clam *Saxidomus giganteus* exerted a hypocholesterolemic effect when fed to chicks. Groups of sixteen 8-day old chicks were maintained for 14 days on either a standard diet, a standard diet supplemented with 1% cholesterol, or a standard diet supplemented with 1% cholesterol and 1% clam sterol mixture. Blood samples were taken from the brachial vein and serum cholesterol levels were measured. Mean cholesterol levels were; basic diet, 157 mg/100 ml; basic + 1% cholesterol, 628 mg/100 ml, basic + 1% cholesterol + 1% clam sterol, 356 mg/100 ml. Idler et al. (1970a) continued studies of this hypocholesterolemic phenomenon with scallop sterols. Added dietary cholesterol (0.5%) significantly increased chick blood serum cholesterol, but the effect was less than that found in earlier experiments; there was no significant elevation of plasma cholesterol when 0.5% scallop sterols were included with 0.5% cholesterol. Scallop sterols alone added to the basic diet resulted in a possibly significant decrease in serum cholesterol. The above experiment was repeated (Idler et al. 1970b) using 1% added sterols. Again the effects were not as great as in the earlier study, but the addition of 1% cholesterol to the basal diet did produce a highly significant increase (28%) in plasma cholesterol level. Scallop sterols added to the basal diet + 1% cholesterol caused a significant decrease in plasma cholesterol relative to the cholesterol-fed group. Again there was no significant increase in plasma cholesterol level when scallop ste-

rols alone were added to the basal diet. It was not established why the effects of feeding cholesterol in the latter two studies were much lower than the effect produced in the first study. The authors feel that the breed of chick, or the basic diet, or both, could have been influencing factors. Even though scallop sterols are a complex mixture of at least 11 sterols, with cholesterol comprising only approximately 30%, no additional sterols were found, by GLC analyses, in the plasma of chicks fed scallop sterols.

Summary

It is important to take into consideration that the separation of sterols is a difficult task, particularly the separation of C_{24}-epimers and cis-trans isomers. Gas–liquid chromatography (GLC) and thin-layer chromatography have substantially resolved this problem and revealed the complexity of sterol compositions in many species of molluscs. Much data obtained before the use of GLC should be considered tentative in light of findings since its introduction. Pioneers of marine sterol research published data on "new" sterols that are now considered to have been mixtures; i.e., conchasterol, meretristerol, chalinasterol, cryonasterol, ostreasterol, polyphenasterol, and pteropodasterol.

The principal classes of molluscs which have been examined are the Amphineura (subclass Polyplacophora), Pelecypoda, Gastropoda, and Cephalopoda. Each class has a certain characteristic sterol composition, but variations do exist. Chitons (class Amphineura) are characterized by the predominance of Δ^7-cholestenol, but recent GLC studies have shown that certain species contain mixtures of Δ^7 and Δ^5-sterols. Pelecypods contain the most varied and complex sterol mixtures found in molluscs. With few exceptions, cholesterol is the principal sterol, but as many as thirteen sterols may be present in one species. Other major pelecypod sterols include 24-methylenecholesterol, brassicasterol, and 22-dehydrocholesterol. Gastropod sterols are similar to pelecypod sterols but trend towards a single sterol, cholesterol. Cholesterol is characteristic of the cephalopods. The quantities of $\Delta^{5,7}$-sterols vary in pelecypods and gastropods, but generally they are minor components.

Some important gaps remain to be filled to give a complete picture of the variety of sterols from this interesting and diverse group of animals, the molluscs. Nothing is known of the sterols of the worm-like molluscs from the subclass Aplacophora (class Amphineura) or the highly specialized pelecypods of the subclass Septibranchia. Two small and primitive classes, the Monoplacophora and the Scaphopoda (tooth shells or tusk shells), which are phylogenetically important, have not been examined for sterols. *Neopilina galathea* is the only known survivor of the Monoplacophora and externally it resembles both a chiton and a gastropod. The pteropods appear to be the only species of the subclass Opisthobranchia (class Gastropoda) examined. In the highly specialized group, the cephalopods, studies have been confined to the cuttlefish. The readily available species of squid, octopuses, and nautili have not been examined.

There appears to be a considerable variation in total sterol content of molluscs, but these variations may be due to numerous factors. In two studies of seasonal variations, total fat, unsaponifiable and sterol contents were lowest in July (oyster) and August (scallop), indicating a transfer of fatty material to the gonads.

Sterol biosynthesis has been demonstrated in many pelecypods and herbivorous gastropods. The carnivorous gastropod *Buccinum undatum* and the cephalopod *Sepia officinalis* were incapable of synthesizing sterols.

Hypocholesterolemic sterols are present in molluscs.

Systematic Nomenclature[2] *and Empirical*	*Formulae*
22-*trans*-24-norcholesta-5,22-dien-3β-ol	$C_{26}H_{42}O$
7-dehydrocholesterol = cholesta-5,7-dien-3β-ol	$C_{27}H_{44}O$
22-dehydrocholesterol = 22-*cis*-cholesta-5,22-dien-3β-ol; 22-*trans*-cholesta-5,22-dien-3β-ol	$C_{27}H_{44}O$
desmosterol = cholesta-5,24-dien-3β-ol	$C_{27}H_{44}O$
cholesterol = cholest-5-en-3β-ol	$C_{27}H_{46}O$
lathosterol = 5α-cholest-7-en-3β-ol	$C_{27}H_{46}O$
cholestanol = 5α-cholestan-3β-ol	$C_{27}H_{48}O$
ergosterol = (24R)-24-methylcholesta-5,7,22-trien-3β-ol	$C_{28}H_{44}O$
brassicasterol = (24R)-24-methylcholesta-5,22-dien-3β-ol	$C_{28}H_{46}O$
pincsterol = (24S)-24-methylcholesta-5,22-dien-3β-ol (?)	$C_{28}H_{46}O$
24-methylenecholesterol = 24-methylenecholest-5-en-3β-ol	$C_{28}H_{46}O$
campesterol = (24R)-24-methylcholest-5-en-3β-ol	$C_{28}H_{48}O$
22:23-dihydrobrassicasterol = (24S)-24-methylcholest-5-en-3β-ol	$C_{28}H_{48}O$
corbisterol = 7-dehydrostigmasterol = (24S)-24-ethylcholesta-5,7,22-trien-3β-ol	$C_{29}H_{46}O$

[2]Sterol nomenclature is in accordance with the IUPAC/IUB. Revised Tentative Rules for Nomenclature of Steroids (Arch. Biochem. Biophys. 136: 13–35, 1970); 24α-alkyl groups are denoted by the prefix "(24R)-24-alkyl." "Introduction of a Δ^{22}-double bond reverses the specification of chirality at $C_{(24)}$; thus, stigmasterol is (24S)-24-ethylcholesta-5,22-dien-3β-ol" (Brooks 1970 p. 75).

poriferasterol = (24R)-24-ethylcholesta-5,22-dien-3β-ol ..... $C_{29}H_{48}O$
stigmasterol = (24S)-24-ethylcholesta-5,22-dien-3β-ol ..... $C_{29}H_{48}O$
fucosterol = 24-ethylidenecholest-5-en-3β-ol $C_{29}H_{48}O$
28-isofucosterol = 24-ethylidenecholest-5-en-3β-ol ..... $C_{29}H_{48}O$
β-sitosterol = (24R)-24-ethylcholest-5-en-3β-ol ..... $C_{29}H_{48}O$
clionasterol = (γ-sitosterol ?) = (24S)-24-ethylcholest-5-en-3β-ol ..... $C_{29}H_{50}O$

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Physiological Responses of Three Species of Fishes to Various Salinities

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BROCKSEN, R. W., AND R. E. COLE. 1972. Physiological responses of three species of fishes to various salinities. J. Fish. Res. Bd. Canada 29: 399–405.

The effects of varying salinity on parameters such as growth, food consumption, food conversion efficiency, and respiration were determined for three species of fish inhabiting the Salton Sea, California. Bairdiella (*Bairdiella icistia*), orangemouth corvina (*Cynoscion xanthulus*), and sargo (*Anisotremus davidsoni*), were subjected to salinities ranging between 29 and 45‰. The optimal range of salinity was between 33–37‰ for all three species. Growth, food consumption, food assimilation, and respiration were adversely affected at the extreme salinities of 29 and 45‰. The results indicate that the fish inhabiting the Salton Sea will experience difficulty in maintaining populations of the current size when the salinity reaches 40‰.

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Les auteurs ont mesuré les effets de diverses salinités sur des paramètres tels que croissance, consommation et efficacité de conversion des aliments et respiration chez trois espèces de poissons de la mer Salton, Californie. Ils ont exposé *Bairdiella icistia, Cynoscion xanthulus* et *Anisotremus davidsoni* à des salinités variant de 29 à 45‰. La gamme optimale de salinité se situe entre 33 et 37‰ pour les trois espèces. Les extrêmes de salinité de 29 et 45‰ ont un effet adverse sur la croissance, la consommation et l'assimilation d'aliments et la respiration. Il semble bien, d'après ces résultats, que les populations de poissons de la mer Salton pourront difficilement se maintenir à leurs niveaux actuels quand la salinité atteindra 40‰.

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THE Salton Sea is the largest inland body of water in California. It presently supports a sports fishery of considerable importance and is utilized heavily by the 8 million residents in the Los Angeles area. The success of fish species introductions into the Salton Sea have usually been limited. However, the introduction of bairdiella (*Bairdiella icistia*), orangemouth corvina (*Cynoscion xanthulus*), and sargo (*Anisotremus davidsoni*), proved successful and have provided a substantial sports fishery. Since the introduction of these three species, the Salton Sea has become progressively more saline because of the lack of a freshening water supply. Salt importation to the Basin has increased from an average of 3.95 million tons per year from 1945 to 1962 to 5.59 million tons per year since 1962. Presently, the salinity of the Salton Sea is 37‰, and it has been predicted that by the year 1975 it will increase to approximately 40‰ (Walker 1961; United States Geological Survey 1969). If this prediction becomes a reality, there will be an obvious physiological stress placed on the fish inhabiting the Salton Sea.

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In seeking to define the tolerable limits to fish of salinity change in the Salton Sea, we believe that such limits should be defined in terms of the production of the fish populations involved. Assuming that the reproduction and activity of the fish populations are not influenced (these questions are being investigated by others), the problem of the influence of increasing salinity on the production of any fish population can be reduced to direct effects on the growth of the fish and effects on their food organisms.

The importance of energy relations to individual organisms, to the success of populations, and to the stability of communities, has been generally accepted by biologists. Ivlev (1961) has suggested quantitative approaches to the study of growth in fishes, and Winberg (1956) and Fry (1957) have contributed much valuable information on the bioenergetics and growth of fishes. Warren and Davis (1967) and Davis and Warren (1968) have further defined terms of Ivlev's bioenergetic model for use in measuring the possible fates of energy consumed.

The data presented in this paper are the results of efforts to quantify the effects of various salinities on the growth, respiration, and assimilation capacities

of bairdiella, sargo, and corvina under laboratory conditions.

Methods and Materials

Growth, assimilation, and respiration of each species were studied at the five salinities of 29, 33, 37, 41, and 45‰, in the spring and summer of 1969. The California Department of Fish and Game facilities at Salton City, California, were utilized.

The juvenile sargo, bairdiella, and yearling corvina used in our experiments were collected with beach seines from the Salton Sea and were transported to the laboratory, where they were held for one week in 250-gallon tanks. The fish were selectively sorted for uniform size (Table 1) and for apparently good physical condition. After sorting, the fish were placed in aquaria for salinity acclimation. During the period of acclimation and also during the experiments, the fish were fed commercially frozen bait shrimp and squid. Salton Sea water used in the experiments was filtered through glass wool and charcoal to remove detritus and other suspended material. Throughout the experimental periods, the salinity of the Salton Sea was 37‰. Lower salinity waters (29‰ and 33‰) were prepared by diluting with distilled water and higher salinities (41‰ and 45‰) by evaporation followed by dilution with distilled water. A conductivity bridge was used to measure salinity. The *p*H and dissolved oxygen content of the water were continually monitored and did not fall in a range where they could be considered a stress factor.

Feeding experiments were conducted in 75 translucent plexiglass aquaria, each having a capacity of 10 liters. This material was used to reduce any behavioral problems such as those inherent in growth experiments conducted in clear aquaria in which fish are held next to one another and fed different rations. Moreover, unfiltered sea water is turbid and it was believed there would be fewer behavioral problems if the visual environment in the aquaria more closely simulated that in the Salton Sea. Evaporation was a problem even though all aquaria had translucent plastic covers. For this reason, salinities were checked daily and adjusted by the addition of distilled water. The aquaria were arranged in five sets of 15 each. Water was recirculated in each set through the use of a headbox, where aeration, filtration of particulate material, and heating occurred. Approximately 20% of the water in the system was freshened each day by introduction of new water in the headbox. Air-conditioners maintained the air temperature at 21±2 C. The water was heated to 25±0.5 C, and day length was held at 15 hr.

Growth and Food Consumption

The growth rate–consumption rate experiments were conducted with juvenile sargo and bairdiella ranging in length from 8 to 10 cm (Table 1). Forty-five fish were gradually acclimated to each of the five test salinities over a 2-week period. The salinity was changed by increments of 1‰ per day until the desired salinity was achieved. Thereafter, the fish were held at the experimental salinity for the remainder of the 2-week period.

TABLE 1. The species and weights of experimental fish used in the Salton Sea investigations.

Test	Salinity (‰)	Species	Test Weight (*g*)	
			Mean	Range
Growth	29	Sargo	1.50	1.06–1.88
	33	"	1.55	1.35–1.84
	37	"	1.59	1.22–2.01
	41	"	1.59	1.12–1.99
	45	"	1.60	1.37–2.07
	29	Bairdiella	4.89	4.15–5.34
	33	"	4.85	4.26–5.83
	37	"	4.89	4.09–5.63
	41	"	5.13	4.28–5.91
	45	"	5.00	4.13–5.92
Assimilation	29	Corvina	16.67	7.70–29.67
	37	"	15.53	3.27–32.98
	45	"	16.00	6.23–26.13
Respirometry	29	Corvina	10.08	8.90–11.45
	33	"	13.72	3.54–24.65
	37	"	17.31	2.95–32.98
	41	"	16.30	3.70–23.89
	45	"	14.73	8.12–23.75

Excess food was available to the fish during the acclimation period. Fifteen fish were selected from the original 45 acclimated to each salinity. These 15 were divided into five groups of three fish each. The groups were individually fed rations of 1, 2, 4, 6, and 10% of their wet body weight. The change in weight of the fish during the test period was calculated on a dry-weight basis. Each value obtained was divided by the mean dry weight of the fish for the time interval involved (14 days) and the quotient was then divided by the number of days of actual feeding in the interval (10 days). Growth rates were expressed in terms of milligrams of growth per gram of biomass per day.

Respiration

Respiration studies were conducted using yearling corvina acclimated to the five test salinities. The respirometers used were circular 2-liter black plexiglass containers through which water could be continuously passed. Water was cycled between the respirometer and a headbox where aeration and temperature control was accomplished. Following introduction and acclimation of the individual fish, an initial water sample was withdrawn. The water source to the test chamber was then sealed off and the animal was allowed to respire in the dark, unfed, for periods of time ranging from 15 min to 1 hr. At the conclusion of the test period, a final water sample was withdrawn. The water samples were analyzed for dissolved oxygen using the Azide Modification of the Winkler method (American Public Health Association 1960). Values were expressed in terms of total oxygen consumed (mg O_2/hr) and oxygen consumption rate (mg O_2/g/hr).

Assimilation

Yearling corvina were acclimated to 29, 37, and 45‰ salinities. After acclimation, a 48-hr period of food deprivation was followed by feeding rations of known weight and caloric value. The fish were held for 48 hr to allow for digestion and assimilation. Then, the resultant fecal material was collected from the aquaria, filtered, and frozen along with a 30-ml aliquot of the filtrate for analysis of organic matter. A wet combustion method was employed using dichromate as the oxidizing agent (Brocksen et al. 1972). The percentage of food assimilated by the fish held at the various salinities was calculated by dividing the milligrams of oxygen required to oxidize the fecal material and other organic material in the sample by the milligrams of oxygen required to oxidize the same amount of food fed.

Results and Interpretation

Food Consumption and Growth of Sargo and Bairdiella

The results of growth rate–consumption rate experiments conducted with juvenile bairdiella at 29, 33, 37, 41, and 45‰ indicate that at the lower feed-

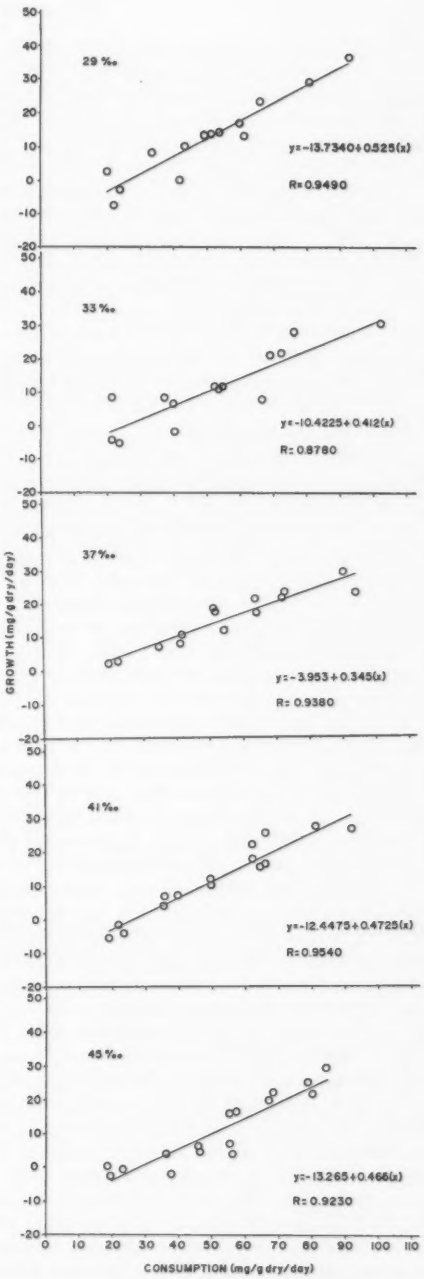

Fig. 1. Relation between consumption rate and growth rate for juvenile bairdiella held at five salinities.

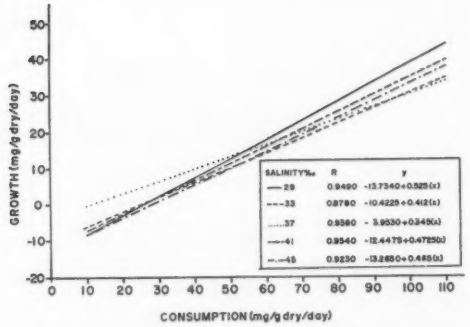

FIG. 2. The influence of salinity on growth rate–consumption rate relationships of juvenile bairdiella.

ing levels the salinity at which the bairdiella is most efficient is 37‰ (Fig. 1, 2). The favorability of the 37‰ salinity is particularly evident at the maintenance level of energy intake where, on a dry–weight basis, 11.16 mg of food per gram dry weight of fish per day (mg/g dry per day) was required to maintain this species in a no-gain, no-loss state (Fig. 1). At a salinity of 41‰, the maintenance requirement increased by 136% to 26.37 mg/g dry per day (Fig. 1) and at the highest salinity tested, 45‰, 28.52 mg/g dry per day was required for maintenance (Fig. 1). The latter figure represents an increase of 156% above the energy required to maintain the fish at the 37‰ salinity. While the bairdiella had a relatively high efficiency ratio at the three lowest feeding levels (1, 2, and 4% of the body weight) at 37‰ they did not utilize their food as efficiently at the highest feeding levels of 6 and 10% of their body weight (Fig. 2). This may be a result of experimental error in collecting uneaten food rations at the higher feeding levels. During these experiments, it was discovered that the possibility of passage of food between individual containers existed and this situation was then corrected while the experiment was in progress. Table 2 gives the maintenance rations at the various salinities for both the bairdiella and the sargo.

The results of the growth experiments with juvenile sargo were quite different from those of the bairdiella (Fig. 3, 4). The most favorable salinity for food utilization appeared to be 33‰ for the sargo (Fig. 3, 4). Sargo held at a salinity of 45‰ were the least efficient, indicating a rather severe stress (Fig. 4). The sargo held at 37‰ were inefficient in utilizing their food for growth (Fig. 3, 4). The sargo being an ocean species has evolved to grow and reproduce in salinities approximating 35‰. The stress indicated by a 2‰ difference is not in proportion to that caused by the other salinities. Considering the osmoregulatory processes involved in maintaining a

TABLE 2. Food (mg/g dry/wt/day) required to maintain bairdiella and sargo in a no-gain, no-loss state (maintenance) at five salinities, and the correlation coefficients derived from the growth rate-consumption rate experiments.

Salinity (‰)	Maintenance		Correlation coefficient	
	Bairdiella	Sargo	Bairdiella	Sargo
29	26.16	75.83	0.949	0.918
33	25.29	63.48	0.878	0.971
37	11.16	77.34	0.938	0.895
41	26.37	75.66	0.954	0.798
45	28.52	78.71	0.923	0.970

rather constant internal environment, one would expect the growth at 33‰ and 37‰ to be nearly the same. The energy required for maintenance of this internal environment would not be expected to be significantly different between 33‰ and 37‰.

The most striking feature of the curves in Fig. 4 is the increase in the amount of energy required to simply maintain the fish. The maintenance ration increased from 63.5 mg/g dry per day at a salinity of 33‰ to 78.7 mg/g dry per day at a salinity of 45‰ (Fig. 4, Table 2). The difference represents an increase of nearly 25% in the amount of energy required to meet the maintenance requirements.

The major difference between the results of the experiments involving the sargo and the bairdiella is the comparative location of the growth curve at 37‰ for the sargo. The shifting of the growth-rate curves to the right is certainly an effect of the stress placed on the fishes by exposure to the different salinities. This is substantiated by an examination of the results of food assimilation and respiration experiments.

ASSIMILATION

The amount of food assimilated by the juvenile corvina from a given ration was highest at the salinity of 37‰ and lowest at the extreme salinities of 29‰ and 45‰ (Table 3). Determinations were not made at other salinities or with other species. We recognize that there can be differences in the amounts of food assimilated from a given food ration between species (Brocksen et al. 1972), but because of logistic problems and time limitations, the number of experiments reported here were limited. We believe that because the three species of fish under consideration are carnivores, the results are valid for comparative purposes. It appears that in relation to the maintenance requirements of the fish, a portion of the increase in food requirements above and below

the 33‰ and 37‰ salinities is probably a result of the decreased food assimilation at the extreme salinities.

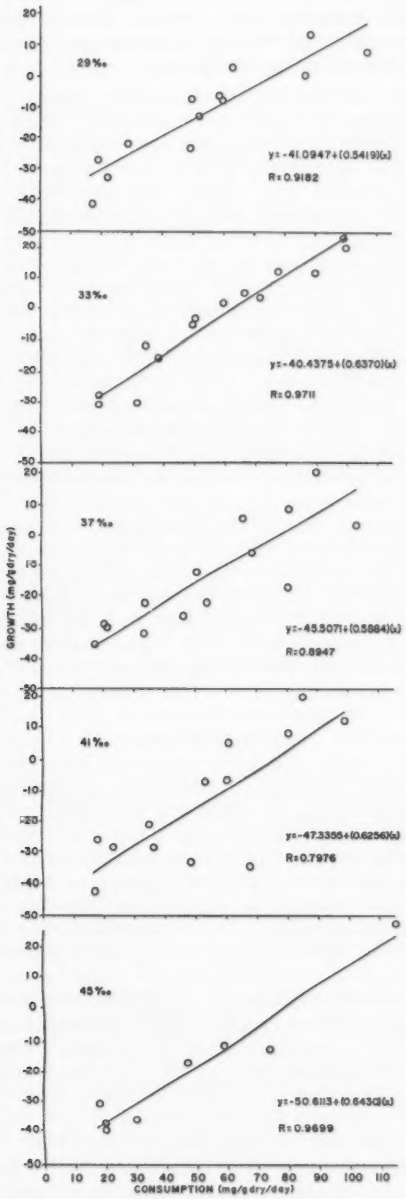

FIG. 3. Relation between consumption rate and growth rate for juvenile sargo held at five salinities.

RESPIRATION

Total respiration is the amount of oxygen consumed by the fish per given unit of time (here expressed per hour) and was measured using yearling corvina of different weights at various salinities (Fig. 5). Oxygen uptake was markedly higher by the fish held at 29‰ and 45‰ than by those fish held at the intermediate salinities. When the oxygen uptake values are divided by the weight of the fish and plotted against the fish weight, the result is respiration rate (Fig. 6). The higher rates of respiration are exhibited at the extreme salinities of 29‰ and 45‰ and the lower rates at the intermediate salinities.

Respiration values obtained in these experiments were not equivalent to the standard metabolism of the fish, but rather are routine metabolism (Fry 1947), because the fish were simply held in darkened containers and activity was not controlled. Control over activity during respiration measurements is desirable when synthesizing energy budgets (Warren and Davis 1967). There is, however, interest in routine metabolism. The convergence of the points at

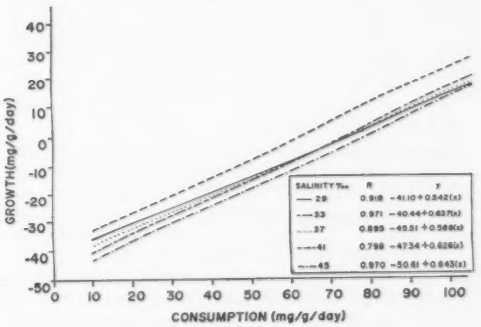

FIG. 4. Influence of salinity on growth rate–consumption rate relationships of juvenile sargo.

TABLE 3. Assimilation efficiencies for Corvina held at 25 C in three different salinities.

Salinity (‰)	Percent Assimilation	
	Range[a]	Mean
29	63–69	66.25
37	70–75	72.00
45	59–60	59.33

[a]Number of determinations in each case was five.

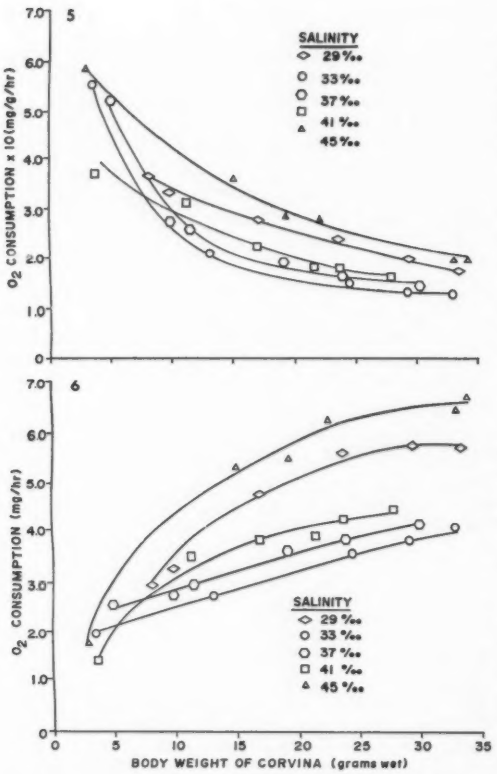

FIG. 5. Respiration rate of the orangemouth corvina at five different salinities.

FIG. 6. Total oxygen consumption of the orangemouth corvina at five different salinities.

the left in Fig. 5 and 6 indicate that the smaller corvina have a different response to the extreme salinities than do the corvina of greater weight. The small corvina are either not as physiologically influenced by the extreme salinities or their activity is markedly suppressed. Only activity control would help to explain the convergence of the respiratory curves with the small fish. It is for this reason that standard metabolism is preferred over routine metabolism as a measure of stress.

Discussion

We did not attempt to investigate all of the energy demanding physiological processes involved in budgeting the energy of a fish. Changes in salinity experienced by the fish could cause changes in specific dynamic action (Averett MS 1969), but this is difficult to measure. Costs of activity and behavior, too, are difficult to measure and are most often estimated as residuals after growth and the other energy-demanding processes are accounted for. In routine investigations of effects of water quality, it is perhaps not necessary to measure either specific dynamic action or activity.

Our ultimate goal for management of fishes of the Salton Sea is knowledge of the effects of increased salinity on their production rates. Since production is most often defined as the product of growth rate and biomass, it is obvious that any decrease in growth rate without a simultaneous increase in biomass will result in a decrease in production. It has been demonstrated by the relations shown in Fig. 1–4 and the data in Table 3, that decreases in growth rate due, in part, to increases in respiratory costs and a decrease in assimilation efficiencies can be expected at salinities above 40‰ and below 32‰. This is especially true with bairdiella at the lower feeding levels. Production is a complex event involving food supply, density of fish, and competition for food (Brocksen et al. 1968, 1970; Brocksen and Warren MS 1972). Without growth, there is no production, and certainly no reproduction, and it is because of this that we believe it is the best single measure for determining the influence of water-quality changes on fish (Averett and Brocksen 1970). The measurements of growth in this study were not made without difficulties, and there are definite seasonal differences which must be taken into consideration. Ideally, growth experiments for each species of fish should be conducted during the four seasons of the year. During the time periods involved in this study, fishes of a similar weight and age were used. In addition, a food ration of uniform quality was used. Because of these procedures, comparative information of sufficient value to define the physiological stress caused by salinities ranging between 29–30‰ and 40–45‰ was derived. Those salinities causing the least stress for the species studied can be defined on this basis as being between 33‰ and 37‰. This range takes into consideration only growth and the processes involved with energy utilization, but not reproduction. It is unfortunate that information on growth, assimilation, and respiration are not available for all three species of fish. This was, in part, owing to availability of fish and, in part, owing to limited facilities. Yet, the corroborative results of the growth data of the bairdiella and the sargo (Fig. 1–4), combined with the assimilation and respiration data derived using the corvina, exhibits some rather basic responses to the stress of salinity. Without all of the aforementioned data for any one species, it is impossible to construct a meaningful energy budget. There is danger in the extrapolation of any

laboratory-derived data to existing wild populations, but the basic principles and relations can often warn us of potential or impending stresses such wild populations may experience. Elucidation and understanding of such causal factors is essential before any sound management programs can be initiated.

If, then, there is a resultant increase in the maintenance requirements of the fishes at salinities other than those in the range of 33–37‰, one wonders if the food base is adequate to meet these needs in the Salton Sea. The pile worm, *Neanthes succinea*, forms the basis of the rather simple food chain existing presently in the Sea (Carpelan and Linsley 1961). This organism must be able to afford the increase in energy requirements to the fish in question if they are to grow and reproduce. If the physiological stress placed on the fishes is being experienced by the pile worm, it is questionable whether or not the worm can provide the increased energy needs or, in fact, absorb the increased pressure and maintain a population of any consequence. We believe, as with the fish, that the measurement of growth and its related processes point the way towards the most meaningful evaluation of the influence of increasing salinities on the pile worm.

Irrespective of the dynamics of the pile worm populations, the bairdiella, sargo, and corvina inhabiting the Salton Sea are presently undergoing salinity stress. We believe the solution to this problem lies in the retardation of increasing salinity in the Salton Sea, for even the most rapid physiological adaptation cannot be visualized for the increasing salinities predicted in the near future.

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Lipid Composition of the Queen Crab (*Chionoecetes opilio*)

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Muscle tissue of the queen crab, *Chionoecetes opilio*, contained 0.75% lipid, 78.8% of which was phospholipid (chiefly choline phosphatides) and the balance mainly cholesterol. Viscera contained 13.6% lipid, the bulk of which was triglyceride. Fatty acid composition was generally similar to that of other marine species. However, 20:5ω3 was the major polyunsaturate (rather than the more usual 22:6ω3) and within the 18:1 series, the ω7 isomer was present in unusually large quantities.

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Le muscle du crabe des neiges, *Chionoecetes opilio*, contient 0.75% de lipides, dont 78.8% sont des phospholipides (surtout des phosphatidylcholines) et la balance, en majorité du cholestérol. Les viscères contiennent 13.6% de lipides, en grande partie des triglycérides. La teneur en acides gras est en général semblable à celle des autres organismes marins. Cependant, le principal acide gras polyinsaturé est 20:5ω3 (et non le plus commun 22: 6ω3) et, dans la série 18:1, l'isomère ω7 se rencontre en quantités exceptionnellement grandes.

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THE work reported here was carried out to establish lipid class distribution and fatty acid composition in the queen (or snow) crab, *Chionoecetes opilio*. This brachyuran crab now forms the basis of a flourishing fishery in the Atlantic Provinces of Canada, but no information about its chemical composition was available previous to a preliminary report of our studies, including proximate composition of crab meals, which has already appeared (Addison et al. 1970).

The lipids of the larger marine crustacea have not been extensively studied (Huggins and Munday 1968). Nevertheless, enough information exists in the literature to indicate that lipids from this group conform to normal marine lipid patterns of class distribution and fatty acid composition (de Koning and McMullan 1966; de Koning 1970; Allen 1971). Some differences may exist, however, in the proportions of lipids or distribution of polyunsaturated fatty acids which might affect flavour, texture, or nutritional value of queen crab relative to other species.

Experimental

Live queen crab, trapped in the Gulf of St. Lawrence and landed at Shippegan, N.B., at various times between October 1969 and January 1970, were frozen and shipped to the laboratory in Halifax. They were stored at –40°C until required for analysis. Dissection of the freshly thawed animals yielded muscle tissue (from legs and "shoulders") and viscera (the balance of the shell contents, including all the internal organs, but chiefly the hepatopancreas).

Tissue samples from several individuals of each lot were pooled (no attempt was made to group the animals by either sex or age) and lipids extracted, transesterified, and the methyl esters analysed by gas liquid chromatography (GLC) as described previously (Addison et al. 1968). Some of the methyl ester sample was hydrogenated, and the resultant saturated acids examined by GLC. A further portion of the methyl esters was fractionated by thin-layer chromatography (TLC) on silver nitrate-silicic acid plates and the monoethylenic esters eluted and analyzed by open-tubular GLC (Ackman and Castell 1966). A further lipid sample was subjected to column chromatography on silicic acid (Addison et al. 1968) and Sephadex LH-20 (Addison and Ackman 1969), and the fatty acids of the major fractions obtained were also analyzed by the above procedures.

Results and Discussion

Lipid contents of samples, and the lipid class distributions, are shown in Table 1. The muscle tissue was relatively lean, and most of the lipid present was phosphatide. Otherwise, the lipid class composition was similar to that observed in fish tissue (Addison et al. 1968), except that the level of sterols (probably mainly cholesterol: Idler and Wiseman 1968) was

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TABLE 1. Lipid content and lipid class distribution in fresh *C. opilio* muscle and viscera.

	Muscle	Viscera
Lipid content (% wet weight)	0.75	13.6
Lipid classes (as % total lipid)		
Hydrocarbons	0.6	1.4
Sterol esters	0.4	91.3[a]
Triglycerides	7.3	
Free fatty acids	3.8	
Sterols	10.1	3.8
Cardiolipin	3.8	trace
Cephalins	22.1	1.1
Lecithins	49.1	2.4
Sphingomyelins	3.8	

[a]Includes a small amount of diacyl glyceryl ethers.

higher in the crab. De Koning (1970) has reported that the marine crab *Cyclograpsus punctatus* contains a small amount of ceramide aminoethyl phosphonate, but we did not attempt to identify this material in *C. opilio* lipids. It is likely that it would be included in the lecithin fraction if present. However, the pooled viscera were quite rich in lipid, most of which was triglyceride. The bulk of this probably originated from the hepatopancreas, which appears to be the major lipid storage organ in crabs (Giese 1966; Allen 1971; Lawrence 1971) and lobsters (de Koning and McMullan 1966; Zandee 1967; Ackman and Cormier 1967). In *Carcinus maenas*, phospholipid is also reported to be the major fraction in muscle lipid, and somewhat surprisingly, in hepatopancreas lipid also (Munn, cited by Huggins and Munday 1968). A band with TLC behaviour similar to that of marine diacyl glyceryl ethers (from dogfish liver oil) was detected in the visceral lipids, but was insignificant in comparison with that for the triglycerides.

Fatty acid distributions in the total lipids of muscle and viscera are shown in Table 2. Muscle lipids (mainly phosphatides) were relatively rich in polyunsaturates and deficient in the longer chain (C_{20} and C_{22}) monoenes, whereas the reverse was true for the visceral lipids (mainly triglycerides). Although the iodine values (calculated from fatty acid composition) were of the same order as those in other marine lipids (Addison et al. 1968), the major polyunsaturated acid present was 20:5ω3[1] rather than the 22:6ω3 characteristic of most marine vertebrate lipids. The presence of relatively large amounts of 20:5ω3 seems to be characteristic of the lipids of the larger decapod crustacea; it has been observed in the rock lobster *Jasus lalandii* (de Koning and McMullan 1966), the Dungeness crab *Cancer magister* (Allen 1971), the blue crab *Callinectes sapidus* (Whitney 1969a), and even (though this animal was not of marine origin) the crayfish (Wolfe et al. 1965). It may also be true of smaller crustacea such as copepods, e.g. *Temora longicornis* (Ackman and Hooper 1970), decapod shrimp, e.g. *Pandalus borealis* (Ackman and Eaton 1967), and euphausiids (Pierce et al. 1969; Yamada 1964; Hansen and Meiklen 1970; Mauchline and Fisher 1969) with the possible exception of *Meganyctiphanes norvegica* (Ackman and Eaton 1967), and a number of decapods and one euphausiid examined by Culkin and Morris (1969). Curiously, some reports of smaller crabs such as *Pleuroncodes planipes* (Pierce et al. 1969) and *Cyclograpsus punctatus* (de Koning 1970) indicate more 22:6ω3 than 20:5ω3. However, the sublittoral *P. planipes* examined in another study showed 20:5ω3 > 22:6ω3 (Lewis 1967). Species living at these relatively shallow depths may show seasonal variations (Jeffries 1967), perhaps mainly affecting hepatopancreas lipid. The deeper-dwelling *C. opilio* might be expected to show a more stable fatty acid composition, especially in muscle lipids. Deep-dwelling species of various types reportedly have high levels of 18:1 (Lewis 1967, compare Culkin and Morris 1969). We found no emphasis on 18:1 in lipids of *C. opilio* which were fished at depths not exceeding 150 m.

The distribution of fatty acids among various individual classes of muscle lipid is also summarized in Table 2. Generally, these were similar to those observed in other species (see Addison et al. 1968 for a review), with triglycerides being rich in monoenes and low in polyunsaturates, lecithins rich in 16:0 and polyunsaturates, and cephalins containing less 16:0 and more polyunsaturates than lecithins. The composition of the free fatty acid fraction was unusual in that it resembled that of the cephalins. However, the cephalins contained more 22:6ω3 and less 20:5ω3. Generally, free fatty acids of muscle are believed to arise through random hydrolysis of phospholipids, but the free fatty acids of crab muscle do not resemble such a hydrolysate (Addison et al. 1969). We are unable to offer any explanation of this at present, except to suggest that this fraction may be derived both from phospholipid hydrolysis and a hypothetical pre-existing metabolic pool of free fatty acids.

Examination of the isomers of the monoethylenic acids by open-tubular GLC (Ackman and Castell 1966) showed an unusual distribution within the

[2]Shorthand notation for chain length (no. C atoms): No. of double bonds, and position of last double bond relative to terminal methyl group.

TABLE 2. Fatty acid composition of *C. opilio* tissues and muscle lipid fractions (as % acids $C_{12} - C_{22}$).

Fatty Acid	Muscle					Viscera (total)
	Total	TG[a]	FFA[a]	PE[a]	PC[a]	
12:0	tr[b]	0.3	0.2	tr	tr	0.2
14:0	0.5	3.8	0.7	0.2	0.5	3.6
14:1	tr	0.4	tr	tr	0.1	0.5
15:0	0.3	0.5	0.3	0.2	0.4	0.5
15:1	tr	tr	tr	tr	tr	0.2
16:0	14.0	13.9	9.6	9.1	17.5	11.8
16:1	5.9	10.4	3.7	3.1	6.7	10.8
16:2ω4	0.8	0.7	0.4	0.9	0.8	0.7
16:3ω4	0.2	tr	0.2	0.7	0.3	0.1
16:4ω1	0.2	tr	0.4	tr	0.4	0.4
17:0	0.6	0.2	0.6	1.0	1.0	0.4
17:1	0.2	tr	0.4	0.3	0.3	tr
18:0	2.4	2.1	2.4	3.5	1.8	1.8
18:1	21.7	23.7	18.1	20.3	22.3	22.6
18:2ω6	0.7	0.7	0.6	1.3	1.1	1.1
18:3ω3	0.2	0.3	0.4	tr	0.3	0.6
18:4ω3	0.1	tr	0.1	tr	0.2	0.4
19:1	0.3	tr	–	–	–	0.1
20:1	2.6	11.9	1.7	1.2	1.5	14.2
20:2ω6	0.1	0.2	tr	tr	tr	0.5
20:4ω6	3.9	1.7	7.9	5.5	3.0	1.4
20:4ω3	tr	tr	tr	–	–	0.4
20:5ω3	30.5	10.7	43.3	32.6	29.1	10.6
21:5ω2?	–	–	–	tr	–	–
22:1	0.6	7.7	tr	–	–	9.4
22:5ω3	0.9	1.4	0.6	0.7	1.1	0.9
22:6ω3	13.3	9.2	8.5	19.3	11.5	6.5
I. V. (Calculated)	229.3	144.0	269.7	264.1	215.0	137.6

[a]TG, triglyceride; FFA, free fatty acids; PE, cephalins; PC, lecithins.
[b]tr, trace.

TABLE 3. Monoene isomers in *C. opilio* tissue lipids (isomers as % of each chain length).

Acid	Total	TG[a]	FFA[a]	PE[a]	PC[a]	Viscera (total)
14:1ω7 (Δ^7)	–	–	–	–	–	–
ω5 (Δ^9)	100	100	100	100	100	100
16:1ω9 (Δ^7)	3.8	4.0	15.2	4.3	5.3	2.0
ω7 (Δ^9)	94.4	92.0	75.7	87.5	90.7	94.1
ω5 (Δ^{11})	1.9	4.0	9.1	8.1	4.0	3.9
18:1ω9 (Δ^9)	49.2	56.3	42.8	32.2	58.1	56.2
ω7 (Δ^{11})	48.0	41.5	53.4	63.4	39.5	41.1
ω5 (Δ^{13})	2.9	2.2	3.8	4.4	2.4	2.7
20:1ω13(Δ^7)	–	–	–	–	–	–
ω11(Δ^9)	12.6	19.8	15.6	13.7	25.0	22.7
ω9 (Δ^{11})	53.3	61.7	53.4	52.9	52.1	52.1
ω7 (Δ^{13})	31.1	18.4	31.1	33.3	22.9	25.2
ω5 (Δ^{15})	3.0	–	–	–	–	–

[a]TG, triglyceride; FFA, free fatty acids; PE, cephalins; PC, lecithins.

18:1 series. Normally the Δ^9 isomer predominates in short (C_{16}) and medium (C_{18}) chain acids, presumably because desaturation at the Δ^9 position is the major route to these acids. In longer chain acids (C_{20} and up), chain lengthening of the shorter C_{16} and C_{18} monoenes assumes greater importance. Usually the ratio of Δ^9 to Δ^{11} isomers in 18:1 in marine vertebrates is about 3:1 or higher (Ackman and Castell 1966; Addison and Ackman 1970), but in crab muscle total lipids the ratio is about 1:1, and in visceral lipids is fairly close to this ratio (Table 3). This indicates that chain lengthening of 16:1 is an important route to 18:1. This ratio, and that among the 20:1 isomers, has been also observed in the phospholipids of the euphausiid crustacean *Thysanoëssa inermis*, but not in *M. norvegica* (Ackman et al. 1970). The activity of the chain lengthening process could depend on the availability of shorter chain monoenes in the diet, and hence the isomer ratio could be diet-dependent as well. However, it is not clear to what extent this would affect the isomer ratios in different lipids. Table 3 shows that the 18:1 of cephalins contains relatively large amounts of the Δ^{11} isomer. Brockerhoff and Ackman (1967) also found relatively large amounts of this isomer in the ethanolamine phosphatides of cod, but not of mackerel. Isomer distribution in the 16:1 and 20:1 acids was roughly similar to that observed in other marine lipids (see references cited above). The 22:1 isomers in the crab phospholipids were difficult to analyze accurately because of their very low overall proportions in the lipids.

Our data indicate that, for the most part, the lipids of this animal are fairly typical of marine lipids. The unusual features of monoethylenic fatty acid distribution are probably explained by peculiarities in the metabolism of crustacea. Thus the presumptive inability of *C. opilio* to synthesize cholesterol and consequent dependence on dietary sources for sterols (Zandee 1967; Idler and Wiseman 1968; Whitney 1969b; Kanazawa et al. 1971) could extend to other metabolic systems and place emphasis on factors from the diet.

Note added in proof: The topic of utilization and disposal of crustacean fishery wastes is discussed in detail by V. Mendenhall in "Utilization and disposal of crab and shrimp wastes," University of Alaska Marine Advisory Bulletin No. 2, College, Alaska, 1971, 41 p., and by S. P. Meyers and J. E. Rutledge in "Shrimp meal – A new look at an old product," Feedstuffs Nov. 27, 1971, p. 31–32. Additional recent lipid and compositional data on crustaceans has been published by R. A. Krzeczkowski, R. D. Tenney, and C. Kelley, "Alaska king crab: fatty acid composition, carotenoid index and proximate analysis," J. Food Science 36: 604–606 (1971), and by J. Van der Veen, B. Medwadowski, and H. S. Olcott, "The lipids of krill (*Euphausia* Species) and red crab (*Pleuroncodes Planipes*)," Lipids 6: 481–485 (1971). The unsaponifiable matter in the liver oil has been identified primarily as cholesterol and α-glyceryl ethers by H. Seino, S. Watanabe, K. Kawada, and Y. Abe, "Study on the liver oil of marine crustacea. I. Sterols and glyceryl ethers in the liver oils of *Chionoecetes opilio* and *Lithodes turritus*," Yakagaku 20: 802–806 (1971).

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Laboratory Rearing of Spot Shrimp Larvae (*Pandalus platyceros*) and Descriptions of Stages[1,2]

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Spot shrimp (*Pandalus platyceros*) were cultured in the laboratory from ovigerous females and eggs detached from females caught in Dabob Bay of Hood Canal, Washington. A tank was designed specifically to incubate the detached eggs in a recirculating seawater system. After hatching, the larvae were cultured in beakers in a water bath and fed newly-hatched *Artemia*.

Morphological descriptions of the larval stages were made and discussed. The fifth stage was considered the megalopa (post-larval).

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Nous avons élevé des crevettes (*Pandalus platyceros*) au laboratoire à partir des femelles ovipares et des oeufs prélevés sur des femelles capturées dans la baie Dabob du canal Hood, Washington. Un bassin spécialement construit a servi à l'incubation des oeufs prélevés dans un système de recirculation d'eau de mer. Après éclosion, nous avons placé les larves dans des bechers au bain-marie et les avons nourries de larves d'*Artemia* nouvellement écloses.

Nous décrivons et analysons les caractères morphologiques des stades larvaires. Le cinquième stade est considéré comme stade mégalope (postlarvaire).

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ALTHOUGH pandalid shrimp form a fairly important fishery on the Pacific coast of North America (58.9 million pounds, $3.2 million Lyles 1967), remarkably little is known about their early life history. Of the five commercially important species (*Pandalopsis dispar*, *Pandalus platyceros*, *P. hypsinotus*, *P. borealis*, and *P. jordani*) only *P. jordani* has been cultured in the laboratory (Modin and Cox 1967).

With special interest in the mariculture field these days, consideration was given to the potential of pandalid shrimp culture along the Pacific coast. Of the five species mentioned above, *P. platyceros* seemed best suited for this possibility because of its large size and apparent rapid growth rate in comparison with related species. The four larval stages of this species that have been described previously were described tentatively from the plankton (Berkeley 1930). Therefore, as part of our attempt to determine the potential of this species for mariculture, the early life history was studied in the laboratory. The objectives of this paper are to present the egg incubation techniques utilized in this study and to clarify the morphological differences between larval stages.

Rearing Methods and Materials

In early January 1967, ovigerous female spot shrimp were caught by shrimp trawl in Dabob Bay, Washington. They were held in styrofoam live-holding boxes and transported to the recirculating saltwater system at the College of Fisheries. As the trawl crushed and killed many ovigerous females, eggs detached from the abdomens of these females were also used for rearing.

To rear larvae, a new recirculating saltwater system was designed incorporating the latest ideas in marine aquarium management (Clark and Clark 1964) and ultraviolet sterilization (Herald et al. 1962). The hatching box (Fig. 1) was similar to one used by Phillips (1971) to hatch eggs of *Palaemon serratus*.

The hatching box was made from plexiglas and consisted of four chambers. Filtered, aerated, and sterilized water flowed into the first chamber and passed through a hole into the bottom part of the second chamber. It flowed up and among the eggs, which were suspended on a curved plastic screen. The flow was adjusted so that the eggs barely moved under the water flow. When the larvae hatched, they swam upward (being positively phototro-

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[2]The work reported here was part of a thesis submitted by the senior author to the Graduate School, University of Washington, in partial fulfilment of the requirements for the Master of Science degree.

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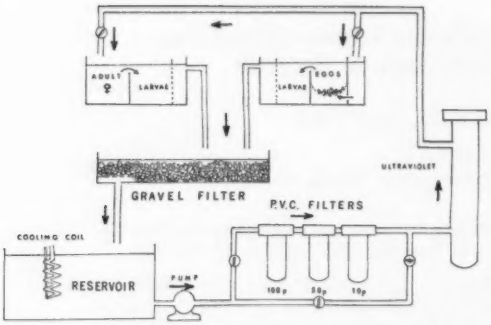

FIG. 1. The recirculating saltwater system. Arrows show direction of flow.

phic) and were carried over the partition into the third chamber. From here they were removed with a large bore pipette. The concave screen shown was more effective than a flat horizontal one. The flat screen allowed eggs to accumulate in the corners, the areas of least flow, and these eggs had a higher mortality. Eggs were held for as long as 60 days without any fungus appearing. The box was used to hatch three species of pandalids and presumably may be used to hatch eggs of other crustaceans.

The recirculating saltwater system (Fig. 1) was constructed entirely from nontoxic materials. All plastics used were "cured" in salt water before use. Pipes and ball valves were ¾-inch polyvinyl-chloride (PVC). The filters were transparent, rigid PVC with wound polypropylene cores of different pore sizes. The adult and hatching boxes were of plexiglas with "Tygon" tubing, and the reservoir was a 50-gal fiberglass tank. The pump had a hard plastic impeller, and the cooling coil was stainless steel covered with a hard plastic sleeve. The ultraviolet (UV) sterilizer consisted of a PVC jacket with two 40-w UV tubes in quartz casings.

Salt water was cooled in the reservoir, pumped thrhoug 100μ, 50μ, and 10μ filters, through the UV unit and into the adult tank and hatching box. A gravity reservoir was eliminated, as it would have caused bacterial recontamination. Overflow from the two tanks flowed through a crushed oyster shell and gravel filter to the reservoir. Airstones aerated and kept water circulating in the reservoir.

The recirculating aquarium system contained 200 gal of water, of which 5 gal were exchanged for fresh sea water each week. Salinity, *p*H, and temperature were checked daily, while bacterial counts were taken every second day. Temperature was kept initially at 7–9 C and later raised to 12–14 C. As the system was in a greenhouse, hot summer days later caused the temperature to rise to 20–22 C. Salinity was kept at 29–30‰ by the addition of distilled water, evaporation being the major problem. Hood Canal water rarely rises above 31‰ (University of Washington, Department of Oceanography 1954), so higher salinities were avoided.

The quality and quantity of light were not controlled; however, direct sunlight was avoided.

Females hatched their larvae mostly at night. Hatching was usually accompanied by a vigorous beating of the pleopods, causing large numbers of larvae to be liberated at intervals. Hatching from a single female continued over a 7–10 day period. No predation of larvae was noted, even though females were not fed during the hatching period. No prezoeae were seen.

Hatched larvae were transferred by large bore pipette to 800-ml beakers with 500 ml of water. The beakers were held in a controlled temperature water bath. Every morning, larvae (10 per beaker) were transferred by pipette to clean beakers with fresh, unfiltered sea water. Larvae were fed newly hatched nauplii of brine shrimp (*Artemia salina*). Larvae lived 11–13 days on stored yolk even with no food provided. However, they fed immediately after hatching if food was offered. Starved larvae ate their dead relatives, but no active predation was noted. The density of brine shrimp was not controlled, but enough were added so that a few brine shrimp existed after 24 hr. An excess of brine shrimp entangled the fine setae of the larvae and prevented feeding. Experimental larvae were fed exclusively on San Francisco Bay *Artemia*. They died if fed Utah *Artemia*.

The larvae did not moult to any degree of success in the recirculating system, but moulted in unfiltered, noncirculating sea water, even if the water was stored for more than a month. All larvae moulted at night. Remarkably, all deaths noted were caused by a failure to complete the moulting process. The abdominal half was shed successfully, but the anterior half remained attached to the mouth parts and pereiopods in these deaths.

Descriptions of Stages

Larvae were held for 83 days and passed through nine stages at an approximate average of 9 days per stage. The fifth, or post-larval, stage was reached after approximately 35 days at 11 C.

Newly-hatched larvae are brightly colored and readily visible. The first four stages swim backwards and upside-down, using the thoracic exopodites. The fifth and following stages swim forwards using the abdominal pleopods. The first stage is positively phototrophic, the succeeding stages less so. The first six stages can easily be distinguished by the shape of the telson.

Illustrations were made from whole larvae, exuvia, and photographs of live larvae. Larvae were dissected under a binocular dissecting scope (WILD M-5). Appendages were mounted in a drop of glycerin on a slide and drawn to scale with a camera lucida. Figures of mouth parts and appendages represent a single appendage from a single individual. For clarity, setules on setae were usually omitted, but spinose setae are shown.

Exuvia stained with carmine were more distinct than unstained exuvia. Exuvia were more reliable in showing segmentation and setation than whole larvae, because the whole larvae sometimes showed details of the next stage beneath the transparent integument. The term "stage" is used here to denote intermoult, as it is the period between successful ecdyses.

Total length was measured from the anterior tip of the rostrum to the posterior tip of the telson. Measurements

were made with a special larval measuring scale provided by the Biological Laboratory, National Marine Fisheries Service, Galveston, Texas. This scale consisted of a sheet of plexiglas with etched lines bent at different angles; thus larvae, which invariably flex at the abdomen, were measured accurately without attempting to straighten them out.

Stage I — 8.1 mm long, easily recognized by immobile eyes (Fig. 2A, C), bright coloration, laterally expanded carapace and abdominal segments.

Most of body covered with numerous orange-red chromatophores. Ends of pereiopods have such bright yellow and brown chromatophores that larva appears luminescent. Mouth parts and some internal organs bright orange-red. Stored yolk visible through carapace in orange-yellow globules.

Rostrum extends to antennal scale, curves strongly downward in front of eyes (Fig. 2C). Dorsal spines, 12–13, first three over eyes being largest. Carapace expanded laterally, 23–25 fine denticles along outer edges (Fig. 2A, B). This character shared only by *Pandalus stenolepis* (Needler 1938). Antennal and pterygostomian spine present, but hidden beneath immobile eyes, visible only on exuvia (Fig. 2B). No supra-orbital spine.

First five abdominal segments laterally expanded, fine denticles along outer edges. Third segment much wider than others.

First antenna, or antennule (Fig. 2D), consists of a tubular basal segment, with distal conical projection, single heavily plumose seta. Conical projection has three simple setae.

Antenna (Fig. 2D) consists of inner flagellum and outer antennal scale. Flagellum has three setae, one short, two long. Antennal scale with four to five joints at distal end, is fringed with 25–26 heavily plumose setae along inner and terminal margins. Long plumose seta at distal outer margin, later becomes a spine. Two-thirds of distance from distal end, a large plumose seta and a smaller simple seta occur. Coxopodite bears spine that persists throughout larval development.

Mandibles (Fig. 2G) have four and five teeth, respectively, between incisor and molar processes.

First maxilla (Fig. 2E) tri-lobed. Proximal lobe, or coxopodite, has five to seven spinose and several plumose setae. Median lobe, or basipodite, bears double row of 12 multi-serrate spines on terminal margin. Endopodite, originating on lateral margin of basipodite, bears three terminal, two lateral plumose setae. Second maxilla (Fig. 2F) has four lobes. Endopodite has eight sparsely setose setae, the bi-lobed coxopodite has seven terminal, one lateral spinose setae on each segment. Basipodite has one lateral, 12–14 spinose setae. Large oval exopodite is fringed with 20–25 long plumose setae.

First maxilliped (Fig. 2H) has barely cleft protopodite with bi-lobed epipodite. Distal part of protopodite, 15–16 small plumose setae present, while proximal part has six to seven longer plumose setae. Endopodite consists of four distinct segments. Exopodite, 1.5 times as long, has three terminal, eight lateral setae.

Second maxilliped (Fig. 2I) has bi-segmented protopodite. Distal segment has single epipodite, the proximal a pediform endopodite and an exopodite with numerous setae. Endopodite consists of five segments, retains shape until fourth stage. Endopodite more robust than shown by Berkeley (1930).

Third maxilliped (Fig. 2J) has endopodite with five distinct segments, and exopodite of same length.

First three pereiopods have naked exopodites; fourth and fifth, no exopodites. First pereiopod ends in simple conical dactyl (Fig. 2K), second in well-formed chela (Fig. 2L), third to fifth (Fig. 2M, N) have well-formed dactyls.

Pleopods, five pairs, visible as small buds. Tail fan has 16 plumose setae (very unusual in Stage I, but confirmed by Berkeley 1930), and enclosed uropods visible as buds.

Stage II — About 10 mm long, uniform light brown, no large chromatophores. Sometimes, just before moult to stage III, colors of third stage show beneath transparent exuvia. Eyes, immobile in first stage, now stalked. Carapace still laterally expanded, although denticles on edges tend to be fewer, finer. Antennal and pterygostomian spines now visible, and prominent supra-orbital spine is present (Fig. 3C). Rostrum has 15 dorsal, three to four ventral spines. Abdominal segments still have fine denticles, but lateral expansions are reduced.

Antennule (Fig. 3B) shows greatest change, having become segmented. First proximal segment laterally expanded at base, with five lateral setae and 8–10 ventrally projecting plumose setae ringing terminal margin. Large spine projects downward from ventral surface, persisting until fifth stage. Second segment has 7–18 ventral setae on terminal margin, while third segment has five to six ventrally projecting setae. Large outer, small inner flagellum on third segment. Outer flagellum has five groups of sensory setae (aestheates) on inner margin; inner flagellum bi-segmented with single apical seta.

Antennal scale (Fig. 3A) has over 30 long, thin, plumose setae along margins. Joints at distal tip are reduced to three, and distal outer seta now a stout spine. Two lateral setae much reduced. Antenna now segmented.

Mandibles (Fig. 3E) have nine teeth each, while maxillae and maxillipeds show little change from first stage.

Pereiopods have grown only in length and exopodites of first three now have setae (Fig. 3F). Pleopods (Fig. 3G) bi-lobed without setae.

Tail fan (Fig. 3D) not changed in shape, but spines stouter and enclosed uropods longer.

Stage III — About 11.0 mm long, as brightly colored as first. Body orange-red, individual orange-yellow chromatophores visible on pereiopods and mouth parts. All denticles from carapace and abdominal segments have almost disappeared. Lateral expansions becoming narrower. Supra-orbital spine still present, and antennal spine shorter than pterygostomian (Fig. 3H). Rostrum has 15–16 dorsal, four ventral spines.

There are eight long setae on inner margin of first segment of antennule. Second segment has two setae, third has small lobe with four setae and seven long ventrally projecting setae (Fig. 3I).

Antennal scale (Fig. 3J) has 36–38 long lateral plumose setae, but all traces of segmentation have been lost. Antenna almost twice length of blade.

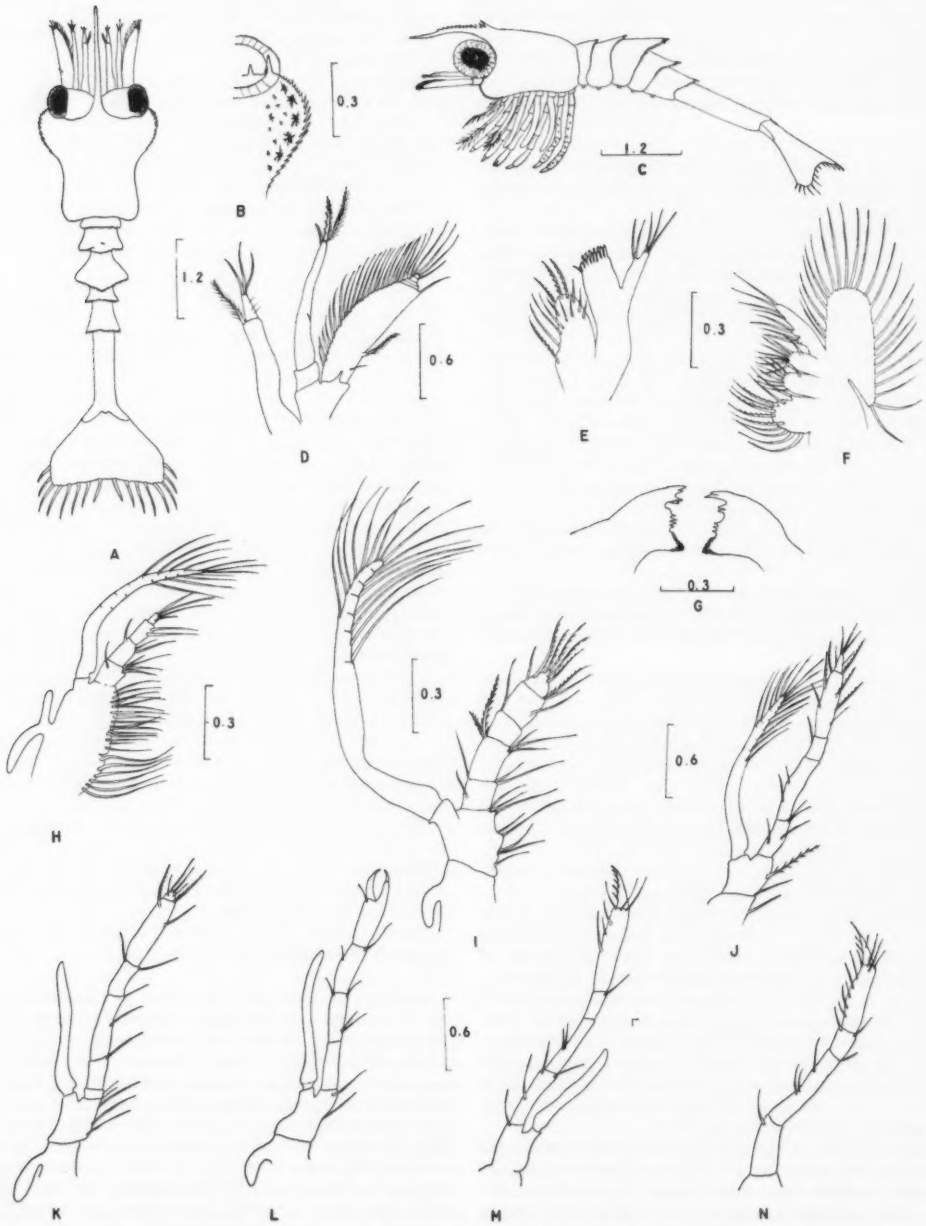

FIG. 2. *Pandalus platyceros* Brandt. Stage I: A, dorsal view; B, carapace margin; C, lateral view; D, antenna and antennule; E, F, first and second maxilla, respectively; G, right and left mandibles; H–J, first to third maxillipeds; K–M, first to third pereiopods; N, fifth pereiopods.

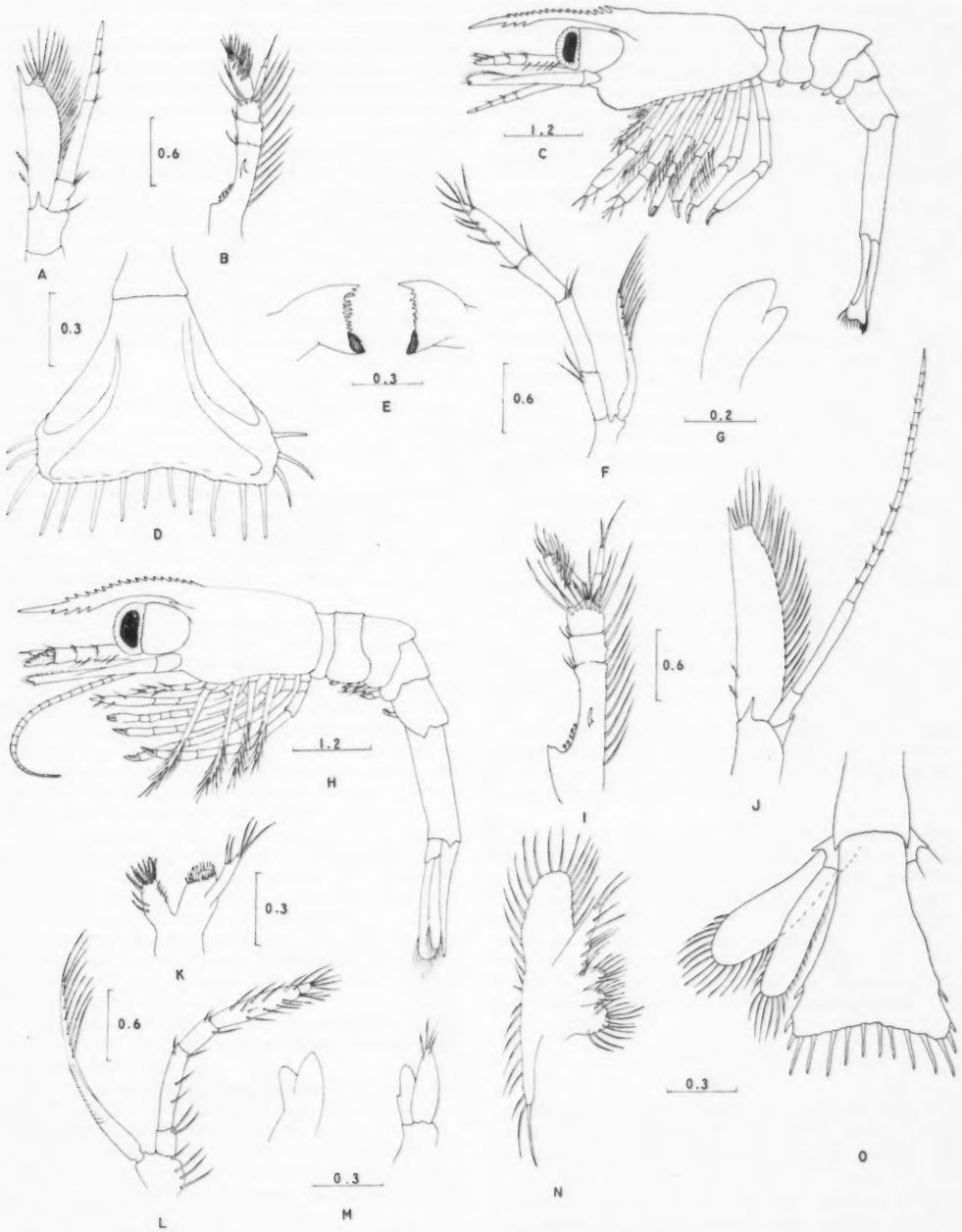

FIG. 3. *Pandalus platyceros* Brandt. Stage II: A, antenna and blade; B, antennule; C, lateral view; D, telson; E, right and left mandibles; F, first pereiopod; G, second pleopod. Stage III: H, lateral view; I, antennule; J, antenna; K, N, first and second maxilla; L, third maxilliped; O, telson.

Coxopodite of first maxilla (Fig. 3K) has seven terminal spinose sefae, as well as three lateral, and five plumose setae. Basipodite has 15 teeth in two rows.

Second maxilla (Fig. 3N) similar to first stage, but proximal part of blade is longer.

Maxillipeds (Fig. 3L) not changed.

Although not shown in the figure, exopodites on pereiopods are much smaller than in previous stages, otherwise do not differ.

Pleopods (Fig. 3M) distincly bi-lobed. First pair have no setae, but second to fifth pair may have two to three setae in precocious individuals, none in others.

Uropods (Fig. 3O) are free. Telson, broader at tip than at base, has 3 pairs lateral, 5 pairs terminal spines.

Stage IV — 11.5–12 mm long, pale brown or greenish hue, small groups of chromatophores remain on base of antennae, eye-stalks, lateral margins of carapace, and pereiopods.

Antennal spine equal in length to pterygostomian, and supra-orbital still present. Rostrum has 15–17 dorsal, five to six ventral spines. Single setae make first appearance between first five dorsal rostral spines (an adult characteristic).

Antennule (Fig. 4A) has bi- or sometimes tri-segmented outer flagellum and bi-segmented inner flagellum with three apical setae.

Antenna 3 times as long as blade (Fig. 4B).

Mandible (Fig. 4C) changed only slightly, and form of fifth stage visible beneath integument.

Maxillipeds and maxillae show little change. Pereiopods not changed, but exopodites decreasing in length. Second pereiopod (Fig. 4D) does not yet have segmented carpus. Margins of abdominal segments have three to four setae each.

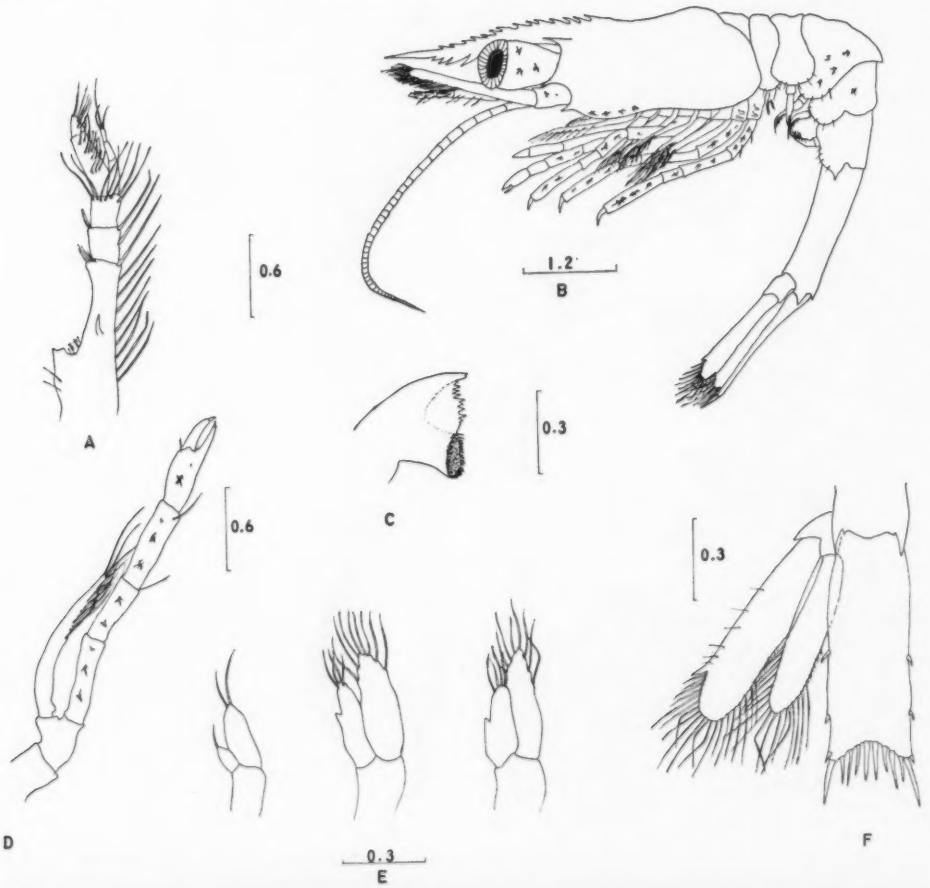

FIG. 4. *Pandalus platyceros* Branpdt. Stage IV: A, antennule; B, lateral view; C, mandible; D, second pereiopod; E, first to third pleopods; F, telson.

Pleopods have well-developed rami. First pair has two setae on exopodite, one on endopodite. Second through fifth have numerous setae, and a bud of the appendix masculina is visible on endopodite (Fig. 4E).

Telson (Fig. 4F) as broad at tip as at base, with four pairs of lateral, four pairs of terminal spines.

Stage V — 12–13 mm, light brown to light green. Scattered chromatophores all over body, but some form distinct spots (Fig. 5D). Carapace has large chromatophore near base of eye-stalk, and a large white spot in hepatic region. There are groups of chromatophores on terminal edges of third to sixth abdominal segments and rostrum. First and second abdominal segments each have a pair of white spots with small chromatophores joining them to form an X when viewed dorsally. Fourth and fifth pereiopods have single large chromatophores on protopodite.

This stage shows most change since first stage. No supra-orbital spine, and antennal spine longer than pterygostomian. Rostrum has 18 dorsal, 6 ventral spines, the ventral spine closest to eye being largest. Single setae between most dorsal rostral spines.

Antennule (Fig. 5B) has lost spine from first segment, and long setae from terminal margin of its third. Outer flagellum has 4 segments, inner has five.

Antenna (Fig. 5A) is $\frac{3}{4}$ length of body, and antennal scale (blade) is assuming square-tipped narrow form of the adult.

Mandibles (Fig. 5F) have incisor and molar processes well separated by a deep cleft, and an unsegmented mandibular palp with four setae.

First maxilla (Fig. 5C) essentially adult. Second maxilla (Fig. 5E) shows some change from fourth stage. Large exopodite almost adult, whereas endopodite is adult and has four setae. Bi-lobed coxopodite is asymmetric; larger distal lobe has 12–14 spinose setae, while proximal lobe has seven. Basipodite has 14–15 mixed spinose and plumose setae on margins.

First maxilliped (Fig. 5G) basically adult. Distal half of cleft protopodite has many short fine setae, while proximal half has seven to eight spinose setae. Exopodite has flattened proximal part (first appearance), and pediform distal part with many plumose setae.

Endopodite of second maxilliped (Fig. 5H) is adult, but exopodite is much stouter. Endopodite has terminal segments bent and flattened with many short spinose setae on lateral margins.

Third maxilliped (Fig. 5I) has lost exopodite, is very heavily setose. Proximal part of merus enlarged, approaching adult form.

All exopodites lost from pereiopods. First pereiopod (Fig. 5L) ends in simple conical segment with ischium articulating somewhat laterally with merus.

Second pereiopod (Fig. 5M) has well-formed chela with joints appearing on carpus for first time. As in the adult, the left second pereiopod is longer, has more segments (10–13) than the right (seven to eight).

Third pereiopod (Fig. 5N) has large stout spines on carpus and merus, but fourth and fifth do not (Fig. 5O).

Margins of abdominal segments have more setae than in fourth stage.

Pleopods (Fig. 6K) are longer, more setose. Appendix masculina, with four to five club-shaped spines, present on second to fifth endopodites.

Telson (Fig. 5P) differs from fourth stage by squarish end and by bearing five lateral, two terminal pairs of spines. Two terminal pairs represented by fine setae.

Since abdominal propulsion is evident by this stage, we may consider this as the megalopa (post-larval). This follows Williamson's (1969) criterion for identifying a post-larval stage.

Stage VI — 14–15 mm long, very light brown, almost translucent. Major groups of chromatophores present from fifth stage are those on rostrum, carapace, and first and third abdominal segments.

Rostrum has 18 dorsal spines, one near tip, and six to seven ventral spines. First ventral spine is large, broad (Fig. 6C).

Antennule (Fig. 6B) has four divisions on outer flagellum, six to seven on inner. Antennal blade (Fig. 6A) essentially adult, with convex outer margin, slender tapering blade, flattened apex. Antenna almost as long as body. Mandibular palp (Fig. 6D) is longer with more setae.

First maxilla (Fig. 6E) has fewer setae on coxopodite, otherwise similar to fifth stage. Second maxilla (Fig. 6F) has longer setae on narrow, distal part of exopodite.

First and second maxillipeds (Fig. 6G, H) similar to those of fifth stage, but third maxilliped has broader merus (Fig. 6I) with heavily setose endite.

First pereiopod has ischium articulating laterally with merus (Fig. 6J), while the second (Fig. 6K) bears more carpal segments. Third to fifth pereiopods all have well-formed dactyls (Fig. 6L) and stout spines on carpus and merus (Fig. 6M).

All pleopods have longer setae; otherwise show little change. Telson (Fig. 6N) shows, for first time, the narrow shape of the adult. It bears five pairs of dorso-lateral spines and two terminal pairs.

Stages VII, VIII, and IX — By the seventh, and especially the eighth stage, mandibular palp is bi-segmented. By the eighth stage, the first pereiopod has fused ischium and merus. Ninth stage (19 mm), telson much more adult, with narrow, tapering tip. Dorso-lateral spines not on dorsal margin as in adult, but become more so in succeeding stages. Pereiopods are striped red and white, as in the adult, but major chromatophore groups of fifth and sixth stages remain.

Discussion

The larval stages of *Pandalus platyceros* reared in the laboratory agree, with two exceptions, with the descriptions of four planktonic larvae by Berkeley (1930). The first stage was identical except that the laboratory-reared larvae had an antennal and a pterygostomian spine (which were visible only on the exuvia) and a segmented antennal scale. Berkeley did not describe a third stage, but her sixth stage was similar to the laboratory-reared fifth stage.

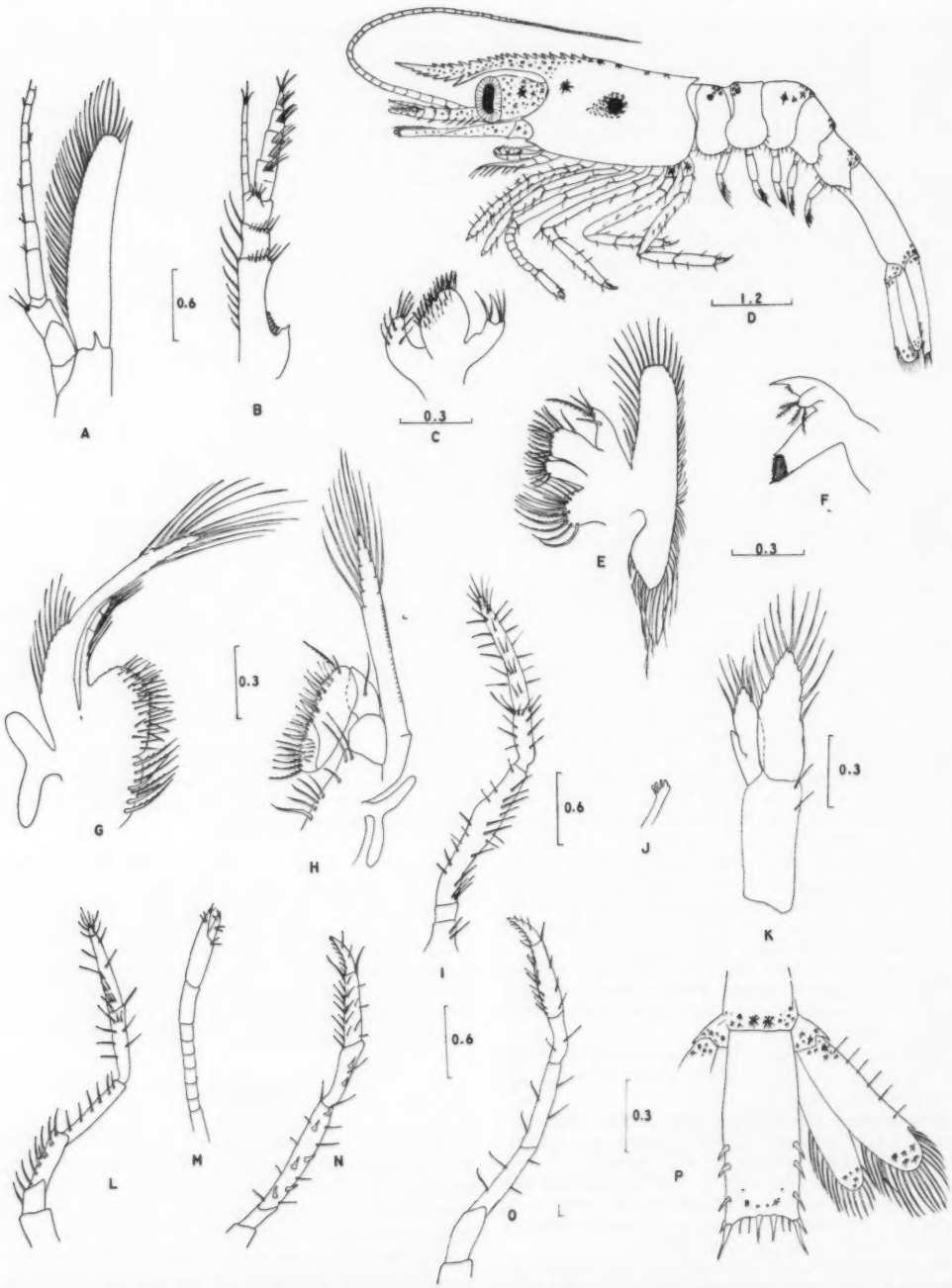

FIG. 5. *Pandalus platyceros* Brandt. Stage V: A, antenna; B, antennule; C, E, first and second maxillae; D, lateral view; F, mandible; G–I, first to third maxillipeds; J, appendix masculina; K, second pleopod; L–O, first to fourth pereiopods; P. telson.

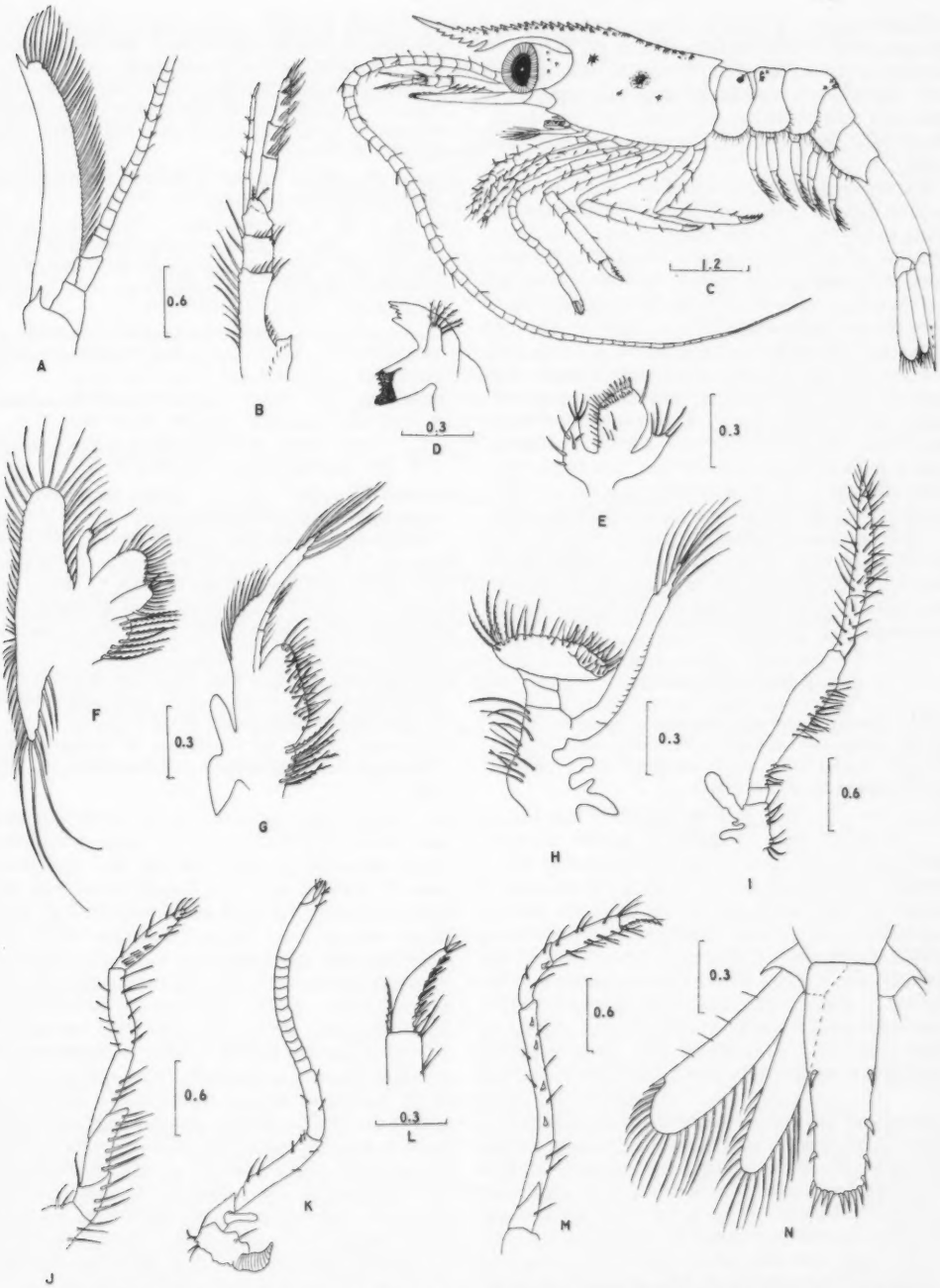

FIG. 6. *Pandalus platyceros* Brandt. Stage VI. A. antenna; B. antennule; C. lateral view; D. mandible; E, F. first and second maxilla; G–I. first to third maxillipeds; J, K, M. first, second, and fifth pereiopods; L. tips of third to fifth pereiopods.

Total lengths of laboratory-reared larvae may not be representative of those found in nature, as laboratory-reared larvae were much smaller than Berkeley's. One fourth-stage larva caught in a plankton haul was similar in morphology, but was much stouter and longer than the laboratory-reared fourth stage.

The segmentation of the antennal scale has been used by Lebour (1940) and Gurney (1942) as a criterion for dividing the first stages of pandalid larvae into two groups. The first group, including *P. montagui*, *P. bonnieri*, *P. stenolepis*, and *P. propinquus*, was characterized by the presence of a jointed antennal scale and rudimentary legs on hatching. The second group, comprised of *P. hypsinotus*, *P. danae*, and *P. platyceros*, had an unjointed antennal scale and functional legs on hatching. Laboratory-reared *P. platyceros* have a jointed antennal scale and functional legs. Kurata (1964) described *P. hypsinotus* with a jointed antennal scale and functional legs. Since the joints on the antennal scale can be seen clearly only on exuvia, it appears that Berkeley could not see the joints on the first stage of *P. platyceros* and *P. hypsinotus*. Thus, until all pandalid larvae have been reared from known parents in the laboratory, Lebour's grouping and Gurney's key should be disregarded.

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Size at Maturity in the Male Alaskan Tanner Crab, *Chionoecetes bairdi*, as Determined by Chela Allometry, Reproductive Tract Weights, and Size of Precopulatory Males

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BROWN, R. B., AND G. C. POWELL. 1972. Size at maturity in the male Alaskan tanner crab, *Chionoecetes bairdi*, as determined by chela allometry, reproductive tract weights, and size of precopulatory males. J. Fish. Res. Bd. Canada 29: 423–427.

An Alaskan Commercial Fishery for tanner crab, *Chionoecetes bairdi*, has recently developed. Only males are legally taken. No size limit exists and the objective of the study was to determine size at sexual maturity as a biological basis for maintaining male brood stocks in adequate numbers. Morphometric measurements, reproductive tract weights, and size of precopulatory males indicate that 50% of the males are mature at about 110-mm carapace width. Using commercial catch size frequency data, the proportion of immature males in the commercial catch was interpolated at less than 3%.

BROWN, R. B., AND G. C. POWELL. 1972. Size at maturity in the male Alaskan tanner crab, *Chionoecetes bairdi*, as determined by chela allometry, reproductive tract weights, and size of precopulatory males. J. Fish. Res. Bd. Canada 29: 423–427.

La pêche commerciale des crabes, *Chionoecetes bairdi*, s'est développée récemment en Alaska. La législation ne permet que la capture des mâles. Il n'y a pas de limite de taille, et la présente étude a été entreprise en vue de déterminer la taille à la maturité sexuelle, fondement biologique du maintien des stocks de progéniteurs mâles à un niveau adéquat. Certaines mensurations morphométriques, le poids des tractus génitaux et la taille des mâles avant l'accouplement démontrent que 50% des mâles sont mûrs lorsqu'ils atteignent environ 110 mm de largeur de carapace. A partir des fréquences de taille des captures commerciales, nous avons estimé à moins de 3% la proportion des mâles immatures capturés commercialement.

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AMERICAN stocks of tanner crabs, which inhabit the Continental Shelf of the North Pacific Ocean and Bering Sea, were first harvested in significant quantities by United States' fishermen in 1968 (Nelson 1969). By that year Japan and the Soviet Union had successfully established tanner crab fisheries in the Bering Sea near the Alaska Peninsula (Pereyra 1967). Domestic Alaskan production increased rapidly from 3.2 million lb live weight in 1968 to 14.5 million lb for the year 1970. The bulk of the catch was from the northwestern Gulf of Alaska (Brown 1971).

Chionoecetes bairdi Rathbun is the only Alaskan commercial *Chionoecetes* species occurring south of the Aleutian Islands or in the Gulf of Alaska (Garth 1958). *Chionoecetes bairdi* probably constitutes over 95% of the Alaskan catch. *Chionoecetes opilio* is fished in the Bering Sea (Takeshita et al. 1969), but only small quantities have been observed in the domestic catch (Kingsbury unpublished data). Although the range of *C. bairdi* includes British Columbia and Washington (Slipp 1952), its North American fishery is presently limited to Alaska.

The purpose of this paper is to discuss male size at maturity in *C. bairdi* in the Kodiak area fishery and the extent to which immature males are being harvested. No commercial size limit for *C. bairdi* exists and biological information is needed to ensure that an adequate male brood stock is maintained. Previous references, to maturity of male *C. bairdi* in Alaska, consist of those of A. P. Kingsbury (unpublished data) and Bright (MS 1967) who dealt briefly with the subject in the Kodiak and Cook Inlet areas, respectively.

Hartnoll (1969) studied mating in brachyuran crabs comparatively, states that "Among Brachyura, external morphology changes affecting, in particular, the chelae may coincide with maturity; these take place at the 'molt of puberty.' However, in males this molt appears to be widely present to a useful degree only in the Oxyryncha, and even then it does not always coincide with the ripening of the gonads." While Hartnoll did not report on Oxyrhynchous ge-

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nus *Chionoecetes*, others have studied male maturity in *Chionoecetes* sp.

Pereyra (1966), who studied reproduction of *C. tanneri* off the Oregon Coast, was able to segregate on sight crabs that had undergone the molt of puberty. Watson (1970) reviewed the Canadian and Asian research on the maturity of *C. opilio* and it's Asian subspecies and presented data on chela allometry and vas deferens weights to establish the maturity size of males in Canada's Gulf of St. Lawrence.

Few publications refer to the capture of mating pairs of *Chionoecetes* sp. Mating pairs of *C. opilio elongatus*, referred to as the "western Pacific cognate" species of *C. bairdi* by Garth (1958), have been observed in tangle nets by Japanese fishermen in the Sea of Japan during late winter and early spring (Kato et al. 1956).

Methods

Chela Allometry

Widths of right chelae and carapaces of 749 male crabs, captured in commercial traps or by trawling from February through May 1970, were measured to the nearest millimeter using vernier calipers. Width of chela was used rather than length primarily because chipped or worn dactyls may reduce length measurements. Chela widths were measured so that caliper jaws were equidistant from the superior marginal spines (Fig. 1). Carapace widths were measured at the maximum width (excluding spines).

Relations between measurements of the various sized crabs were compared to determine if changes occurred at any particular size.

Reproductive Tract Weights

Reproductive tracts from 293 males used in the chela allometry study were weighed to the nearest 0.1 g using an Ohaus 2610-g balance. Carapace widths were compared with reproductive tract weights for each crab. Dissection generally followed that described by Powles (MS 1968). As with *C. opilio*, the testes of *C. bairdi* also merge gradually with and are not readily distinguished from the vasa deferentia in all developmental stages. To facilitate uniformity, the testes and vasa deferentia (reproductive tract) were dissected and weighed together without blotting, after allowing blood to drain.

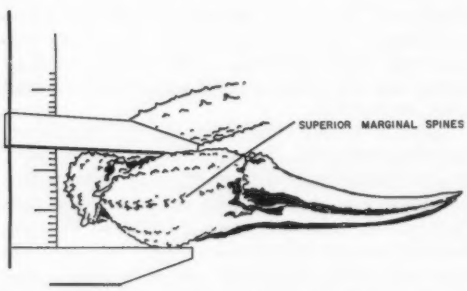

FIG. 1. Measurement of right chela width of tanner crab.

Grasping Pairs

Male tanner crabs select female partners and grasp them prior to copulating in a manner similar to *C. opilio* (Watson 1970). Intact pairs of grasping tanner crabs have been captured by scuba divers in the Marmot–Chiniak inshore waters of the Kodiak area since 1966. Tanner crab pairs were collected in conjunction with the capture of grasping pairs of king crabs, *Paralithodes camtschatica* (Tilesius), by the method reported by Powell and Nickerson (1965). Visibility ranged from approximately 0.5 to 10 m. Measurements of carapace width were taken to the nearest millimeter, using vernier calipers.

Results

Chela Allometry

Means of chela widths were computed for each 1 mm of carapace width (Fig. 2). A distinct break in the data occurring at approximately 110 mm indicates the average size at the molt of puberty.

Least squares regression lines were computed separately for each of the two resulting groups and produced the following:

$$< 110 \text{ mm Carapace width } y = -4.47 + .164x$$
$$\geq 110 \text{ mm Carapace width } y = -19.04 + .315x$$

A Student's t-test indicated a significant difference in the slopes of the regression lines ($P < 0.001$). Important to note is the practical significance in the slope difference since the large sample size and resulting low variance ($3.56 \times 10{-}6$ and $.54 \times 10{-}6$) would produce statistical significance even when the difference in slopes is small. We also note a significant difference in the intercepts. Thus it is clear that we are dealing with two different regression lines.

Reproductive Tract Weights

Of the 293 reproductive tracts sampled, all but nine (discussed below) were examined during the 1970 mating season. Tract weights ranged from less than 0.1 to 14.3 g. The logs of the means of tract weights were plotted for each millimeter of carapace width (Fig. 3). The break in the data occurs at approximately 113 mm and the construction of separate least-squares regression lines produces the following:

$$< 113 \text{ mm Carapace width } y = -5.05 + 0.048x$$
$$\geq 113 \text{ mm Carapace width } y = -0.54 + 0.009x$$

Again the difference between slopes and between intercepts, although small, is statistically significant.

All crabs had hard shells. Reproductive tract weights were compared to chela widths and it was found that not all crabs with mature tracts had yet undergone the molt of puberty. Conversely not all crabs that had undergone the puberty molt had yet acquired mature reproductive tract weights.

Grasping Pairs

Forty-eight mating pairs of tanner crabs have been captured during the past 5 years between the dates of February 21 and May 7. Diving has also been conducted during other months. A few pairs have been observed during mid-January but none later than May 7. Thus, we consider the mating season to be

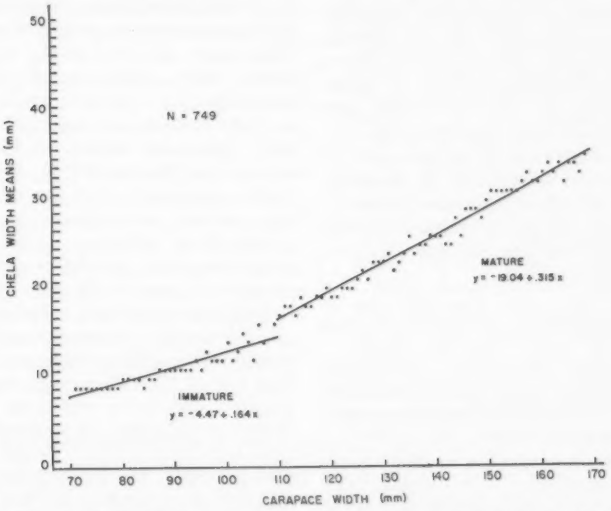

FIG. 2. Relation between size of right chela and carapace width of male tanner crabs, February–May 1970 Kodiak area, Alaska (mean chela widths plotted for each millimeter size group).

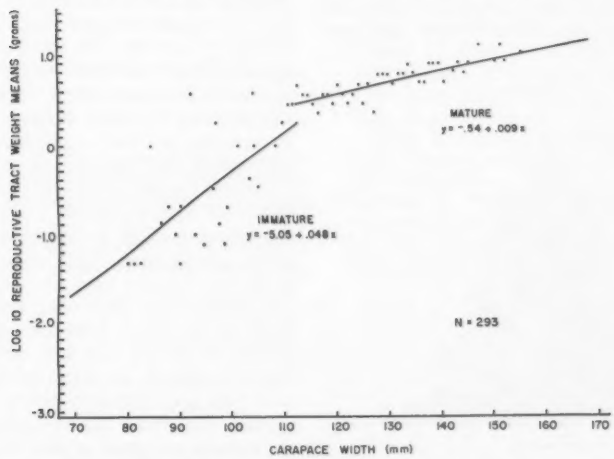

FIG. 3. Relation between reproductive tract weight and carapace width of male tanner crabs, February–March 1970 Kodiak area, Alaska (mean weights plotted for each millimeter size group).

from early January through mid-May. Forty-one of the pairs were captured during 1969. All pairs were captured at depths of less than 25 m (relatively shallow for this species).

Male graspers were generally small, ranging from 93- to 163- mm carapace width. Mean carapace width was 116 mm with 85% smaller than 128 mm. In all cases males were at least 14 mm larger than their female partners; mean size difference being 36 mm.

The corresponding females ranged from 70- to 100-mm (mean 80-mm) carapace width. All but one were nonovigerous oldshell crabs in the premolt stage of ecdysis with internal orange eggs ready for ovulation. We believe all but one of these females were preparing to copulate for the first time and were therefore approaching the molt of puberty. Average size at which females attain maturity is about 90-mm carapace width. Of special interest was the largest female. This crab was the only one with fully developed eyed eggs, indicating she had copulated during a previous mating season.

Proportion of Immatures in Commercial Catch

From the chela and reproductive tract results, the proportion of mature crabs was estimated. With few exceptions, all males attained maturity between 90- and 140-mm carapace width.

To obtain information on the size of males in the commercial catch, 3000 crabs, in 60 landings from 22 vessels were sampled in approximate proportion to the commercial catch from the various Kodiak subareas during the first half of 1970. Mean carapace width was 154.8 (Fig. 4).

Using maturity and size frequency data the proportion of immature crabs within each size category was interpolated. From these data we estimate that less than 3% of the tanner crabs harvested commercially from the Kodiak area during the first half of 1970 were immature.

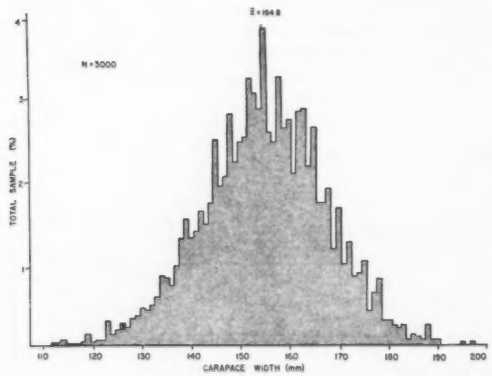

FIG. 4. Size-frequency distribution of male tanner crabs sampled from commercial catches, January–June 1970 Kodiak area, Alaska.

Discussion and Conclusions

The distinct breaks in the lines (Fig. 2, 3) result from disproportionate increases in chela width and tract maturity within the population. Both chela width and tract weight studies yielded similar results with discontinuous points occurring at approximately 110 mm and 113 mm, respectively.

The structure and stages of the vasa deferentia and testes were similar to that of *C. opilio* described by Powles (1968). We did not describe individual tracts in this way or check each one for the presence of spermatophores. While such a procedure is indicated to determine maturity in individual crabs, population maturity size, the chief objective of our study, was nonetheless achieved.

Male graspers were relatively small averaging 39 mm smaller in carapace width than nonmating males in the Kodiak commercial harvest during the first half of 1970. One reason for the size difference may be that larger males are more abundant at depths greater than 50 fathoms (92 m), where the bulk of commercially landed crabs are caught (Brown 1971). Another reason may be mesh selectivity of commercial pots, with smaller males escaping. We assume that large tanner crabs mate at depths greater than that surveyed by divers or in different locations, otherwise they would have been observed on the diving grounds. We further conclude that the sample included some of the smallest males capable of mating. The smallest male grasper captured (93-mm carapace width) approximated the size of the smallest mature males in both the chela and reproductive tract studies. Thus, the results of the grasping pair study compliment the results of the chela width and reproductive tract studies.

Using a regression of chela length against carapace width for a small sample of males and noting the size of our smallest male grasper crab, A. P. Kingsbury (unpublished data) estimated maturity size at 90- to 110-mm carapace width in the Kodiak area. Bright (MS 1967) who studied king and tanner crabs in the Cook Inlet area stated that male *C. bairdi* of 98- to 116-mm carapace length were sexually mature. In terms of carapace width this would be approximately 108 to 130 mm. By comparison, we found the 50% maturity size of males at 110-mm carapace width. We also estimate the range for attainment of maturity at 90- to 140-mm carapace width. Thus, the male crabs found to be mature in the respective studies by Kingsbury

and Bright are also within the maturity size range of our study.

The minimum carapace width of males at maturity for the closely related *C. o. elongatus* was reported to be 66 mm in Korean waters (Yoshida 1941), and 50% of the *C. opilio* males were reported to be mature by 57 mm in the Canadian Atlantic (Watson 1970). Because *C. bairdi* is a larger species, its larger maturity size may be attributed to the species itself. The largest Kodiak area male and female measured have been 207- and 127-mm carapace width, respectively.

Captivity molting data presented by Bright (MS 1967) indicate *C. bairdi* has many age classes in the fishery and may require many years to attain full growth. Thus, if our rapidly expanding fishery becomes depleted through overutilization, as evidenced with *C. opilio* near Japan (Kon et al. 1968), it may require a long recovery period. For economic reasons involving meat extraction, processors in Alaska presently discourage the landing of tanner crabs smaller than 5-inches (125-mm) carapace width. Should meat extraction from smaller crabs become economically feasible, however, State regulatory measures would be indicated to prevent depletion of male breeding stocks.

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Bacterial Population of Diets for Aquarium Fishes

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TRUST, T. J., AND V. G. MONEY. 1972. Bacterial population of diets for aquarium fishes. J. Fish. Res. Bd. Canada 29: 429–433.

Twenty-five commercial diets formulated and offered for feeding to aquarium fish were examined to determine their microbial burden. The total count of aerobic mesophilic bacteria in 24 of the feeds ranged from 9×10^2 to 1.3×10^6 per gram of diet. Twenty of the diets contained from 7×10^2 to 2.4×10^5 bacteria per gram able to grow anaerobically. One of the feeds was sterile. Species from the genus *Bacillus* were most numerous, but species of *Clostridium* (including *C. perfringens*), enterococci, and members of the Enterobacteriaceae were also present in significant numbers. The fish diets supported the growth of a wide variety of bacteria known to be pathogenic to fish and to man.

TRUST, T. J., AND V. G. MONEY. 1972. Bacterial population of diets for aquarium fishes. J. Fish. Res. Bd. Canada 29: 429–433.

Les auteurs ont mesuré la charge microbienne de 25 aliments commerciaux pour poissons d'aquarium. Dans 24, le nombre total de bactéries mésophiles aérobies allait de 9×10^2 à 1.3×10^6 par gramme de nourriture. Ces aliments contenaient, par gramme, de 7×10^2 à 2.4×10^5 bactéries capables de croître dans des conditions anaérobies. Un des aliments était stérile. Les espèces du genre *Bacillus* étaient les plus nombreuses, mais les espèces de *Clostridium* (y compris *C. perfringens*), les entérocoques et les membres des Enterobacteriaceae étaient aussi présents en nombres appréciables. Ces aliments favorisent la croissance d'une grande variété de bactéries pathogènes pour les poissons et l'homme.

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IT is well established that many human and animal feeds carry a bacterial load (Thatcher and Clark 1968). Because foods and feeds have served to distribute bacteria inimical to man and animals, limits have been established for the numbers of pertinent categories of microorganisms tolerable in various foods (Powers et al. 1970; Statutory Orders and Regulations 1955; Thatcher 1963). Diets offered for commercial fish production have recently been shown to contain bacteria potentially harmful to man and to fish (Trust 1971), but microbiological standards have not been established for these feeds. These diets are not generally used to feed smaller aquarium fishes such as guppies (*Lebistes reticulatus*) and goldfish (*Carassius auratus*). The raising of goldfish and other warmwater aquarium fishes in the United States during 1969 represented a business of $12 million (Bullock et al. 1971). Because these fish are used extensively in a number of areas of biological research and are also common household pets, other feeds designed specifically for these fish have been developed and are offered commercially. This communication is intended to make workers using these feedstuffs aware of the microbial burden of the diets. If reports the presence of a wide variety of viable aerobic and anaerobic bacteria in diets available for the feeding of aquarium fishes.

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Materials and Methods

Twenty five different diets were purchased from commercial outlets in Victoria, B.C. These were products of 11 manufacturers and were produced in Canada, Norway, West Germany, Taiwan, and the United States. Twelve of the diets were dehydrated (diets 1–12) and three were in the wet form (diets 13, 14, 15). Also tested were ant eggs (diet 16), *Tubifex* worms (diets 17, 18), *Daphnia* (diet 19), mosquito larvae (diet 20), brine shrimp (diets 21 to 23), brine shrimp eggs (diet 24), and a dried turtle feed (diet 25).

Standard bacteriological sampling techniques were followed in the procurement of samples. Using the procedures outlined in "Standard Methods for the Examination of Water and Wastewater" (American Public Health Association 1965), a 10-g sample was transferred aseptically to a sterile blending jar containing 90 ml of 0.1% (w/v) peptone water and blended at high speed for 2 min. Duplicate dilutions were prepared in 0.1% peptone water. The viable mesophilic bacteria present were enumerated by the Surface Plate Method

recommended by the International Standards Organization (Thatcher and Clark 1968) and by the Drop Plate Method of Miles and Misra (1938). Standard Methods agar (BBL) plates were incubated aerobically at 37 C for 24 hr and anaerobically for 48 hr. Samples of feeds were examined for fluorescin-producing pseudomonads by plating on Pseudomonas F agar (BBL), and for pigment-producing species of *Aeromonas* using Furunculosis agar (Difco) incubated at 30 C. Feed samples were also examined for coagulase-producing *Staphylococcus* by incubating plates of Vogel and Johnson agar (BBL) at 37 C. Selenite F broth (BBL) and Tetrathionate broth (BBL) enrichments at 37 C followed by plating on Salmonella-Shigella agar (BBL), Brilliant Green agar (BBL), and Deoxycholate Citrate agar (BBL) were employed to detect *Salmonella*.

The procedures of the American Public Health Association (1965) were followed for Most Probable Number (MPN) estimates. The MPN estimation of Enterobacteriaceae employed Enterobacteriaceae Enrichment broth (BBL). Fecal coliforms were confirmed by separate loop transfers from the above positive tubes to MacConkey broth (Difco) and incubation at 44±0.5 C. The MPN estimation of *Clostridium* employed Differential Reinforced Clostridial medium (Collins 1967). Presumptive enterococcus estimates employed Ethyl Violet Azide broth (BBL) and were confirmed by separate loop transfers from positive presumptive tubes to fresh Ethyl Violet Azide broth. Additional tests used to partially characterize isolates were those described by Skerman (1967). An agar containing 3% (w/v) fish diet (diets 1–5) was used to test the ability of a number of bacterial species to grow using the diet as sole source of nutrients. Anaerobiosis was obtained using BBL Anaerobic jars with Disposable Hydrogen–Carbon Dioxide Generator envelopes (BBL). Taxonomy was based on the keys of Skerman (1967) and Breed et al. (1957).

Results

The results in Table 1 show that 24 of the 25 aquarium feeds tested contained viable mesophilic bacteria. Diet 21 (Norwegian brine shrimp) was sterile. The number of viable aerobic bacteria demonstrated in the other 24 feeds varied from 9×10^2 to 1.3×10^6/g of feed with a mean value of 1.5×10^5/g. Bacteria able to grow anaerobically were found in 20 samples. The counts of anaerobes were from $< 10^3$ to 1.7×10^5/g with a mean of 3.8×10^4/g. Diet 19 (*Daphnia*) and Diet 24 (brine shrimp eggs) both contained more than 10^5 aerobes per gram and more than 10^5 viable anaerobic bacteria per gram of feed.

Enterobacteriaceae were detected in 23 of the feeds and the MPN estimates were from 40 to 11,000/10 g of diet with a mean of 1023. Seventeen of the feeds containing Enterobacteriaceae contained coliforms and eight contained fecal coliforms. The MPN estimates of coliforms in these diets had a mean of 990/10 g and the MPN estimates of fecal coliforms ranged from 40 to 1500/10 g of feed with a mean of 324. Although Salmonellae were not detected in the feeds, Diet 21 (brine shrimp eggs) did contain significant numbers (9.8×10^3/g) of a species able to grow on Salmonella-Shigella agar. This species was biochemically related to the *Enterobacter* group, but serologically related to the Salmonellas. Agglutination was obtained with *Salmonella* polyvalent "O" antiserum, *Salmonella* Group C_1 antiserum and with *Salmonella* Vi antiserum (BBL). Enterococci were present in 17 of the feeds with MPN values from 40 to 110,000/10 g of feed and the mean was 7352. Diet 7 (goldfish feed), diet 15 (a wet diet), and diet 21 (brine shrimp eggs) contained the largest numbers of bacteria having possible faecal origin.

Clostridium species were detected in 17 diets. The MPN estimates varied from 40 to 110,000/10 g with a mean of 28,404/10 g of feed. Proteolytic species of *Clostridium* were detected in 13 feeds (diets 2, 3, 5, 8, 9, 10, 11, 12, 13, 14, 15, 18, 24). *Clostridium perfringens* was isolated from four feeds (diets 3, 12, 13, and 18). Species of *Bacillus* were the predominant organisms in the diets examined. *Staphylococcus*, *Pseudomonas*, and *Aeromonas* species were not detected nor were any molds.

In addition to demonstrating and enumerating the bacterial load of the fish diets, it was important to ascertain the ability of these fish diets to support bacterial growth. The bacterial species tested wese common pathogens of man or fishes, or common spoilage bacteria. All 5 media containing 3% (w/v) fish diet supported the growth of the following species of bacteria: *Aeromonas liquefaciens* (ATCC 14715), *Aeromonas hydrophila* (9071), *Aeromonas salmonicida* (14174), *Chondrococcus columnaris* (23463), *Clostridium tetani*, *Clostridium perfringens* (3624), *Clostridium sporogenes* (319), *Mycobacterium marinum* (927), *Mycobacterium fortuitum* (9820), *Proteus vulgaris* (13315), *Pseudomonas aeruginosa* (15442), *Pseudomonas fluorescens* (13525), *Pseudomonas putida* (12633), *Salmonella typhi* (167-P), *Salmonella typhimurium* (19585 B), *Shigella sonnei*, *Staphylococcus aureus* (6538), *Streptococcus pyogenes*, *Streptococcus faecalis* (8043), and *Vibrio anguillarum* (19264).

Discussion

The International Committee on Microbiological Specifications for Foods of the International Association of Microbiological Societies has recommended that foods be examined for total viable mesophilic bacteria, staphylococci, Salmonellas, and for coliforms. The Committee has also suggested that foods be examined for species of Clostridia, for anaerobic bacteria, and for enterococci (Thatcher and Clark

1968). The rationale for this choice of organisms and for the microbiological standards presently established for foods has been based on the participation of these bacteria in the spoilage of foods and their involvement in food-borne epidemics and in food poisoning. With fish feeds other considerations are important. The fish using these diets are often held in water at temperatures up to 30 C (Axelrod and Schultz 1969). Because the feed itself is a source of nutrients required for bacterial growth and contains a significant bacterial inoculum, the water in the aquarium tanks may become an ideal bacterial culture medium. In this study, only one of the feeds examined was sterile. This diet had been canned. The numbers of bacteria detected in the other feeds probably represent only a minimum estimate of the total number of viable cells present. Since bacterial action and bacterial growth are nearly directly proportional to a temperature rise from 21 to 30 C (Axelrod and Schultz 1969), the size of the bacterial inoculum in the feed is of great importance.

A wide variety of bacterial species able to cause disease of man and of fishes are also able to grow using the feed as sole source of nutrients. Such bacteria are also mesophilic and could be added to the tanks with the fish, through handling of the tank, or in the feed. Although no Salmonellas were detected in the diets studied, the presence of species of Enterobacteriaceae in many of the feeds does indicate a potential for contamination of such feedstuffs with enteric pathogens. These diets are often handled by children and could cause infection by direct ingestion or by indirect transfer of the organisms to the mouth or respiratory passage via the hands, water, tank sand, or pebbles. Such transfer leading to Salmonellosis has occurred from aquaria housing tortoises (Boy-

TABLE 1. The bacterial load of aquarium diets.

	No. bacteria ($\times 10^3$) per g of feed		Most probable no. of bacteria per 10 g of feed				
Diet	Aerobes[a]	Anaerobes[a]	Enterobacteriaceae[b]	Coliforms[c]	Faecal coliforms[d]	Enterococci[e]	Clostridia[f]
1	0.9	ND[g]	430	430	ND	230	ND
2	29	6.2	40	40	ND	40	43
3	50	9	90	90	40	930	110,000
4	80.5	2.4	430	430	ND	430	ND
5	1.4	2.5	ND	ND	ND	230	1200
6	3.6	ND	90	ND	ND	ND	ND
7	36	16	4600	2400	150	110,000	2400
8	3	ND	40	ND	ND	70	430
9	47	7	40	40	ND	2400	15,000
10	204	12.2	90	90	90	430	11,000
11	38	117	40	40	ND	ND	110,000
12	79	36	40	ND	ND	ND	1500
13	21	5.2	90	90	ND	90	2300
14	49.7	8.4	930	930	150	40	930
15	149.3	39.2	2400	430	430	4600	110,000
16	730	15.6	230	230	140	2400	ND
17	6	ND	40	40	ND	230	ND
18	19	4	40	ND	ND	ND	430
19	27.4	16.4	230	ND	ND	ND	230
20	1,338	244	2100	ND	ND	ND	4600
21	ND	ND	ND	ND	ND	ND	ND
22	2.8	0.7	40	40	ND	ND	ND
23	63	21.7	280	280	90	2400	ND
24	528	170	11,000	11,000	1500	230	110,000
25	96	21.2	230	230	ND	230	2800

[a]Standard Methods agar, 37 C.
[b]Enterobacteriaceae Enrichment broth, 37 C.
[c]MacConkey broth, 37 C.
[d]MacConkey broth, 44 C.
[e]Ethyl Violet Azide broth, 37 C.
[f]Differential Reinforced Clostridial medium, 37 C.
[g]ND is none detected.

cott et al. 1953). Because of the potential ability of bacteria to multiply in an aquarium, consideration must also be given to the risk of skin and wound infections. Aquarium-borne skin lesions have been reported (Månnson et al. 1970). In addition, many of the diets examined contained species of *Clostridium* (including *C. perfringens*) and members of this genus have the ability to cause skin and wound infections.

Workers using fish which require commercial diets must be aware of the presence of this variety of bacterial species in these diets since the food and its bacterial flora will contribute to the fishes' environment. These bacteria have the potential ability to affect the levels of dissolved oxygen, the ammonia concentration, and the hydrogen ion concentration and so may influence the research findings. Furthermore, Bisset (1946, 1948) showed that goldfish (*Carassius auratus*), which were not normally harmed by many saprophytic bacterial species present in aquaria when water temperatures were below 15 C, did succumb to infection by these bacteria when the water temperature is raised. This led to an increased fish mortality due to septicaemia. Many other bacteria which are normally considered saprophytic, including species of *Bacillus*, have been isolated from infections of tropical fish (Almeida et al. 1968). Species of Enterobacteriaceae and strains of enterococci similar to *Streptococcus faecalis* have also been implicated in fish infections and mortalities (Bullock et al. 1971). Species of *Bacillus*, Enterobacteriaceae, and enterococci were detected in the diets examined in this study.

In addition to their use as foods for common aquarium fishes, *Tubifex* worms (e.g. diets 17, 18) have been fed to trout fry (Fiedler and Samson 1935) and coho salmon (Warren and Davis 1967). Brine shrimp (e.g. diets 21–24) have been fed to young trout (Seale 1933) and are routinely used to feed the larvae of sole (*Solea solea*) and plaice (*Pleuronectes platessa*) (Shelbourne 1970). Shelbourne (1970) reported heavy flatfish larval mortalities when *Artemia* eggs from Utah were used instead of those from the San Francisco area and proposed that this was a nutritional failure. The present study suggests, however, that this larval mortality might have been caused by bacteria carried on the brine shrimp eggs. Furthermore, the Scottish workers are rearing the flatfish in a tank complex that is irrigated by heated sea-water from a thermal power station. In future, these flatfish may be raised in such warmwater tanks on a much larger scale for human consumption. Because fish have been shown to be vectors of bacterial diseases of humans (Janssen 1971), it is important that such bacteria are not present in diets of fish being raised for human consumption.

Research workers interested in controlling the fishes' environment and reducing the number of variables influencing research results must give thought to the use of sterile feeds. Various sterile solid diets have been developed that satisfy the dietary requirements of other laboratory animals (Reddy et al. 1968). Consideration must be given to the development and evaluation of sterile feeds for fish. Such diets will have to be used before we can fully understand the advantageous and inimical contributions of bacteria to fish growth.

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Effect of Salmon Gonadotropin on In Vitro Maturation of Oocytes of a Catfish, *Heteropneustes fossilis*

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SUNDARARAJ, B. I., S. V. GOSWAMI, AND E. M. DONALDSON. 1972. Effect of salmon gonadotropin on in vitro maturation of oocytes of a catfish, *Heteropneustes fossilis*. J. Fish. Res. Bd. Canada 29: 435–437.

Salmon gonadotropin failed to induce in vitro ovulation of catfish oocytes at concentrations of 1–40 μg/ml. This observation is consistent with the view that gonadotropin may not act directly on the ovary of catfish in inducing ovulation.

SUNDARARAJ, B. I., S. V. GOSWAMI, AND E. M. DONALDSON. 1972. Effect of salmon gonadotropin on in vitro maturation of oocytes of a catfish, *Heteropneustes fossilis*. J. Fish. Res. Bd. Canada 29: 435–437.

La gonadotropine de saumon n'a pas réussi à déclencher l'ovulation in vitro d'oocytes de silure à des teneurs de 1–40 μg/ml. Cette observation s'accorde avec l'hypothèse que la gonadotropine, en déclenchant l'ovulation, peut ne pas agir directement sur l'ovaire d'*Heteropneustes fossilis*.

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OVINE luteinizing hormone (LH), which induces ovulation and spawning when administered into gravid catfish, *Heteropneustes fossilis* (Sundararaj and Goswami 1966), is only marginally effective under in vitro conditions (Goswami and Sundararaj 1971a). Similarly, the catfish pituitary homogenate, also a potent in vivo ovulating agent (Sundararaj and Goswami 1968), is negative under in vitro conditions over a wide dose range (Goswami and Sundararaj 1971a). It was felt that inasmuch as LH is a purified hormone preparation, and the catfish pituitary homogenate is just a crude extract, gonadotropin present in the latter may be very small. Partially purified salmon pituitary gonadotropin (SG-G100) is 2.5 times more potent than LH in inducing maturation and ovulation in *H. fossilis* under in vivo conditions (Sundararaj and Anand 1972; Sundararaj et al. 1972). It was of interest to investigate if SG-G100 would be more effective than LH in inducing maturation of *H. fossilis* oocytes under in vitro conditions.

Materials and Methods

Specimens of *H. fossilis* were collected during the spawning season (July–August) and maintained as described earlier (Goswami and Sundararaj 1971a, b). Female catfish with enlarged ovaries were quickly killed by decapitation. Small pieces of ovarian tissue, each containing 60–80 yolky oocytes were excised and cultured in 15-ml vials containing 2.5 ml of the culture medium plus various concentrations of SG-G100. In the control vials, ovarian pieces were immersed in the medium without the hormone. All culture vials were shaken periodically on an automatic shaker connected to the mains by a time switch. Culture was carried out for 15 hr (7 PM–10 AM) at 25 ± 1 C.

The culture medium used was a modified version of Wolf and Quimby amphibian culture medium and was prepared by omitting vitamins, amino acids, whole egg ultrafiltrate, and fetal calf serum from the original formulation (Wolf and Quimby 1962; Rothstein et al. 1965). The details of preparation of the culture medium were described earlier (Goswami and Sundararaj 1971a).

The fractionation and purification of salmon (*Oncorhynchus tshawytscha*) pituitary gonadotropin were described earlier (Donaldson and Yamazaki 1968; Yamazaki and Donaldson 1968; Donaldson et al. 1972). The various concentrations of this hormone employed in the present study were dissolved in a constant volume (0.02 ml) of the culture medium.

At the termination of culture, ovarian pieces were removed from the vials. The oocytes were freed from the ovarian tunica, examined under a Leitz stereomicroscope, and classified into four categories (Goswami and Sun-

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dararaj 1971b) according to their stage of maturation: oocytes with germinal vesicle intact, oocytes with germinal vesicle broken, intrafollicular mature oocytes with a differentiated animal pole, and ovulated mature oocytes. Oocytes in each of the four stages of maturation were counted and expressed as percentages of the total numbers of oocytes present in the ovarian pieces. Since the mere breakdown of the germinal vesicle without differentiation of an animal pole (oocytes in second category) cannot be regarded as a reliable index of maturation, only those oocytes that possessed both of these criteria (intrafollicular and ovulated oocytes) were considered mature.

Results and Discussion

Up to concentrations of 10 μg/ml, SG-G100 induced no ovulation in the oocytes (Table 1). A few oocytes showed maturation (5% intrafollicular oocytes) at a dosage of 20 μg/ml, but the response was highly variable. None of the oocytes had ovulated. At 40 μg/ml the response was negative. Ovarian pieces cultured in modified Wolf and Quimby medium without the hormone (control group) did not show maturation.

SG-G100 is a potent in vivo ovulating agent in the goldfish, *Carassius auratus* (Yamazaki and Donaldson 1968). In the catfish *H. fossilis*, a single injection of 50 μg of SG-G100 induces maturation, ovulation and spawning of a large number of oocytes (Sundararaj and Anand 1972; Sundararaj et al. 1972), whereas under in vitro conditions a concentration as high as 20 μg/ml produced only a meagre effect. Similarly, LH (100–250 μg/fish), which is highly effective in vivo in inducing ovulation (Sundararaj and Anand 1972), is only marginally effective under in vitro conditions (Goswami and Sundararaj 1971a). Kirshenblat (1959) also reported that LH was ineffective in inducing maturation and ovulation in vitro in a loach, *Misgurnus fossilis*. On the other hand, corticosteroids such as desoxycorticosterone (DOC) and hydrocortisone (F) are effective in inducing maturation and ovulation under both in vivo (Sundararaj and Goswami 1966) and in vitro conditions (Goswami and Sundararaj 1971a). Further, Goswami and Sundararaj (1971a) have shown that corticosteroids (DOC and F) induce significant in vitro maturation and ovulation of *H. fossilis* oocytes in concentrations (1–5 μg/ml) lower than those of LH. The marginal maturation response obtained in vitro with LH and SG-G100 may be due in part to their ability to induce steroidogenesis in the follicle cells. In this context it is relevant to mention that several of the key enzymes involved in steroidogenesis have been demonstrated in fish ovaries (Bara 1965; Lambert MS 1969; Lance and Callard 1969). These observations are consistent with our earlier suggestion (Sundararaj and Goswami 1969) that gonadotropins such as SG-G100 and LH may not act directly on the oocytes in catfish, but via the interrenal and corticosteroids. The ability of LH to promote corticosteroidogenesis in catfish interrenal slices has already been demonstrated (Sundararaj and Goswami 1969). Experiments are now in progress to determine whether or not SG-G100 induces in vitro corticosteroidogenesis from catfish interrenal.

TABLE 1. Percentages of *H. fossilis* oocytes in various stages of maturation after being exposed to various concentrations of salmon gonadotropin in vitro.

Concn dosage (μg/ml)	Oocytes with germinal vesicle intact	Oocytes with germinal vesicle broken	Intrafollicular mature oocytes	Ovulated mature oocytes
1	92±4[a]	8±4	0	0
	(6)	(6)	(6)	(6)
2	98±1	2±1	0	0
	(6)	(6)	(6)	(6)
5	95±1	5±1	0	0
	(6)	(6)	(6)	(6)
10	94±2	6±2	0	0
	(6)	(6)	(6)	(6)
20	94±4	1±0.6	5±4	0
	(4)	(4)	(4)	(4)
40	93±5	7±5	0	0
	(2)	(2)	(2)	(2)
0	92±2	8±2	0	0
	(6)	(6)	(6)	(6)

[a]Mean ± SE of mean. Numerals in parentheses are numbers of catfish donors from which ovarian pieces were taken for culture.

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Effects of Temperature, Food, and Starvation on Several Physiological Parameters of the Lobster *Homarus americanus*

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STEWART, J. E., G. W. HORNER, AND B. ARIE. 1972. Effects of temperature, food, and starvation on several physiological parameters of the lobster *Homarus americanus*. J. Fish. Res. Bd. Canada 29: 439–442.

The change in physiological condition of groups of fed and starved lobsters, *Homarus americanus*, held for 3–8 months at different temperatures was determined. Changes in muscle content and serum protein measurements were strictly temperature dependent in the case of the starved lobsters. Hemolymph nonprotein nitrogen and lactic acid, hepatopancreatic glycogen, lipid, weight, and moisture content varied directly with food, or temperature, or both; hemolymph carbohydrate values apparently vary more directly with temperature than with food quantities.

STEWART, J. E., G. W. HORNER, AND B. ARIE. 1972. Effects of temperature, food, and starvation on several physiological parameters of the lobster *Homarus americanus*. J. Fish. Res. Bd. Canada 29: 439–442.

Nous avons mesuré les changements de l'état physiologique de groupes de homards (*Homarus americanus*) nourris ou privés de nourriture à différentes températures durant 3 à 8 mois. Les changements qui se produisent dans le contenu du muscle et dans les protéines sériques sont exclusivement fonction de la température dans le cas des homards soumis au jeûne. L'azote non protéique et l'acide lactique de l'hémolymphe, ainsi bien que le glycogène, les lipides, le poids et le pourcentage d'humidité hépatopancréatique, varient en fonction directe de la nourriture et ou de la température; les quantités d'hydrates de carbone de l'hémolymphe semblent varier plus directement avec la température qu'avec les quantités de nourriture.

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THE high mortality rate caused among captive lobsters, *Homarus americanus*, by periodic outbreaks of gaffkemia (Stewart and Rabin 1970), is an ever present and serious economic threat to the lobster industry. Holding captive lobsters at reduced temperatures is one way to reduce the severity of these epizootics, since the mean time to death is extended proportionately by reductions in temperature (Stewart et al. 1969a). The extension of the mean time to death for infected lobsters reaches a point at 1 C where the infection is subpatent.

At present, lobsters are held for considerable periods with little knowledge of the physiological effects on the animals. Among the problems that relatively long periods of captivity for any animal brings are those connected with feeding; feeding is particularly difficult and time consuming when aquatic animals are housed in recirculating systems. Accordingly, in an effort to provide the information required for making reasonable decisions as to choice of temperature, permissible periods of starvation, and the problems connected with feeding, we have measured a number of physiological parameters at several different temperatures. These measurements were made to determine, in addition to the above, which of the several parameters chosen were directly affected by food supply and could be used as possible indicators of the adequacy of synthetic diets.

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Methods

Healthy, vigorous lobsters of both sexes captured at Eastern Passage (near Halifax) were used throughout this study. The large chelae were immobilized with rubber bands immediately upon capture, and the lobsters were weighed and distributed within 24 hr, 24 to each 90 × 90 cm tank, filled to a depth of 35 cm with filtered seawater (Stewart and Power 1963). The water flow rate permitted two changes per hour and the water was maintained within ± 0.5 C of the test temperatures. Experiments at 1 and 3 C were begun in November and continued for 250 days. Those at 5, 10, and 15 C began

in March or April and were terminated after 102 days. Certain groups of lobsters were starved while others were fed each Monday, Wednesday, and Friday, a diet which alternated between herring and beef liver, each given in excess. Surplus food was removed 24 hr after each feeding. Individual shelters were provided for each lobster (Stewart and Squires 1968).

Protein concentrations were determined on lobster serum (Stewart et al. 1966) by Robinson and Hogden's modification of the biuret method (Layne 1957) with bovine serum albumin (Pro-Sol Standard Scientific Supply Corporation, New York, N.Y.) as the standard.

Each 10-ml sample of hemolymph drawn from each lobster was mixed, immediately upon removal, with 500-mg sulfosalicylic acid and centrifuged. The protein-free supernatant solution was used for the determination of carbohydrate (using glucose as the standard) (Morris 1948), lactic acid (Barker and Summerson 1941), and nonprotein nitrogen by the micro-Kjeldahl method using a selenium catalyst.

Glycogen and lipid values were obtained by following the protocol outlined in Stewart et al. (1969b) and using for the actual glycogen analysis the alcohol precipitation and phenolsulfuric acid method of Montgomery (1957).

The total muscle content for both large chelae plus the abdomen of each lobster was determined according to the method described previously (Stewart et al. 1967).

Results

The values for both serum protein and muscle content (Table 1) for lobsters held for the specified periods at the temperatures listed, demonstrate again the close relation between these parameters.

Ten other lobsters selected at random from each fed and starved group held at 5, 10, and 15 C for 102 days were used for determining mean values for hepatopancreatic glycogen, circulating total carbohydrates, lactic acid concentration, and nonprotein nitrogen (Fig. 1A–D). Total lipid content of the hepatopancreatic tissue of these same lobsters are given in Table 2 together with the values for the hepatopancreas as a percentage of live body weight. The results for animals held at 1 and 3 C for 250 days are given in Tables 1–3 for additional information, and were obtained for starved controls selected at random from the experiment at 1 and 3 C described in Stewart et al. (1969a). The conclusion of both experiments coincided.

A 95-day experiment was then conducted, utilizing serum protein measurements alone, to determine the rate at which the change in the physiological con-

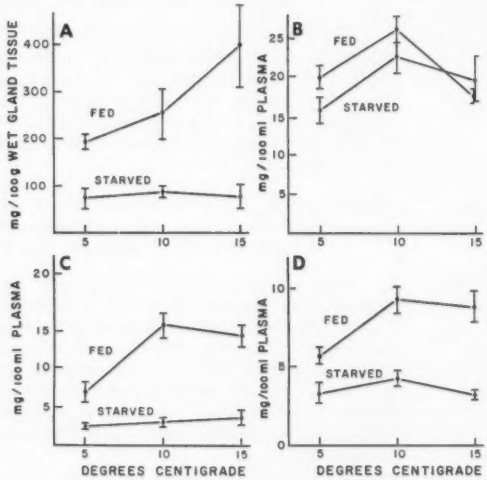

FIG. 1. A, hepatopancreatic glycogen (wet weight basis); B, total hemolymph carbohydrates; C, hemolymph lactic acid; D, hemolymph nonprotein nitrogen (NPN) values, plus standard error, determined for 10 lobsters at each temperature after 102 days of feeding or starvation.

TABLE 1. Muscle content and serum protein concentrations for lobsters[a] held at different temperatures.

Temp (C)	Food	No. lobsters	Duration of experiment (*Days*)	Serum protein (*mg/ml*)	Muscle content (*% of live wt*)	Live weight (*g*)	
						Initial	Final
1	None	19	250	23.9±5.1	17.4±1	564.6±20.5	557.4±20
3	None	20	250	23.1±4	18.3±0.8	555.4±20.3	539±21
5	None	25	102	27 ±3.3	18.3±0.8	520.2±17.5	518.4±17.2
	B/H[b]	25	102	50.3±3.7	23.8±0.7	579.4±30.1	582.1±32
10	None	25	102	21.5±3.3	18.1±0.8	507.1±19.4	506.8±19.5
	B/H[b]	25	102	66.6±3.2	24.4±0.5	565.5±21	575.4±22
15	None	14	102	14.6±3	13.2±1.1	600.1±46.7	602.21±49.8
	B/H[b]	11	102	68.3±4.8	27.1±0.7	594.6±52.4	608.6±53.2

[a]Standard error given for all measurements.
[b]B/H, Beef liver and whole sardine herring given on alternate feeding days.

TABLE 2. Total lipid content of hepatopancreatic tissue.[a,b]

Temp (*C*)	Food	No. lobsters	Final avg wt (*g*)	Duration of experiment (*days*)	Hepatopancreas as % of body wt (*wet wt basis*)	Hepatopancreas % moisture	Hepatopancreas % lipid (*dry wt basis*)
1	None	10	559.9±21	250	3.40±0.25	72.8±2.9	42.9±6.4
3	None	10	534.2±20	250	3.20±0.30	75.2±3.7	34.1±8.8
5	None	10	607.3±69	102	3.30±0.25	69.6±2.9	45 ±5.5
	B/H[c]	10	648 ±64	102	4.74±0.18	58.8±2.9	59.4±4
10	None	9	551.4±52	102	2.27±0.14	80.4±1.7	15.6±4
	B/H[c]	10	626.8±50	102	5.25±0.11	55.6±1.4	64.1±1.9
15	None	10	604.4±52	102	1.98±0.19	79.1±1.5	16.9±4.4
	B/H[c]	10	605.2±33	102	4.93±0.14	52.4±1.6	65.1±2.4

[a]Serum protein values for others in these same groups of lobsters are given in Table 1.
[b]Standard error given for all measurements.
[c]B/H, Beef liver and whole sardine herring given on alternate feeding days.

TABLE 3.[a] Hemolymph total carbohydrates, lactic acid, and hepatopancreatic glycogen plus standard error for lobsters starved for 250 days at 1 and 3 C.

Temp (*C*)	Hepatopancreatic glycogen mg/100 g tissue (*wet wt*)	Total carbohydrates mg/100 ml hemolymph	Lactic acid mg/100 ml hemolymph
1	39.9±5.43	29.5±2.95	2.96±0.8
3	115.6±52.1	34.7±2.5	4.28±0.9

[a]The identical starved lobsters for which other values at these temperatures are given in Table 2.

dition of the lobsters occurred at 5, 10, and 15 C. The results are presented in Fig. 2.

Discussion

Measurements of hepatopancreatic glycogen (Fig. 1A) indicate high values for animals fed beef liver and herring, and relatively low values for starved lobsters. An incidental observation was that two lobsters approaching ecdysis had hepatopancreatic glycogen values as high as 800 mg/100 g of the tissue (wet weight basis). An earlier paper (Stewart et al. 1967) was in error, because on the basis of histochemical evidence alone, we suggested that carbohydrates probably were not an important energy reserve in this animal. This study indicates that glycogen is present in the hepatopancreas in considerable amounts and is an important energy reserve.

The decline in hepatopancreatic proportions with increasing temperatures, taken together with the increase in moisture content, was quite marked among the starved animals (Table 2). The decline, upon starvation, of hepatopancreatic lipid reserves was of the same order of magnitude as that for serum protein and hepatopancreatic glycogen (Tables 1 and 2). Declines in glycogen occur rapidly whereas the depletion of lipid is much slower (Stewart et al. 1969b).

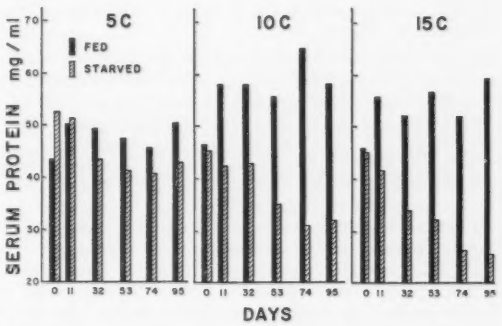

FIG. 2. Hemolymph serum protein measurements made over a 95-day period using groups of 24 fed and 24 starved lobsters at each temperature.

In general, the other parameters showed that values were high for those fed and low for those starved. One exception was the circulating carbohydrate values at 5 and 10 C that were not greatly different between the fed and starved groups, but were essentially the same at 15 C for both groups. An unexplained feature was that circulating carbohydrate and nonprotein nitrogen values were high at 10 C, but low at 5 and 15 C. Also, the weights of the animals did not change significantly during the trials (Table 1).

Temperature as a factor generally appears to have produced lower values for glycogen and lactic acid among the fed animals held at 5 C. The different temperatures did not result in differences in the hepatopancreatic glycogen values, hemolymph lactic acid, or nonprotein nitrogen concentrations among the starved animals. This is attributable to the rather lengthy period of starvation. If measurements had been made during the experiment, the results probably would show that the rates of decline were directly dependent upon temperature, i.e., similar to the serum protein results (Fig. 2). The lobster has the ability to endure long periods of starvation, which we concluded earlier (Stewart et al. 1967). Prolongation of a constant low temperature may be a more serious negative factor than starvation (Stewart et al. 1969a), although, as shown in Table 3, the values for glycogen and carbohydrates among lobsters starved for much longer were not greatly different from those kept at higher temperatures for a shorter period.

The foregoing data indicate that if the temperatures are kept low enough the declines in many physiological factors during the course of a starvation regime are small enough to be tolerated commercially for short-term live storage. Thus, the problems associated with feeding in captivity can be avoided completely except in the immediate postmolt period. The lower temperatures, of course, confer the added benefit of reduction in the hazards of gaffkemia (Stewart et al. 1969a).

The data, additionally, provide evidence as to which of a number of physiological parameters vary directly with food supply and could be used as indices of nutritional adequacy of experimental diets, i.e., serum protein, nonprotein nitrogen, hepatopancreatic glycogen, lipid, and moisture.

Acknowledgments

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Clostridium botulinum in Sediments from the Canadian Atlantic Seaboard

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LAYCOCK, R. A., AND A. A. LONGARD. 1972. *Clostridium botulinum* in sediments from the Canadian Atlantic seaboard. J. Fish. Res. Bd. Canada 29: 443–446.

The presence of *Clostridium botulinum* types B, C, and E has been detected off the coast of Nova Scotia. Whereas types B and E were found only in low concentration on the Scotian Shelf, localized areas of high concentration of type C were found inshore in the Halifax–Dartmouth area and offshore in the deeper waters of Emerald Basin and the edge of the Shelf. Type E only was found off the coast of Newfoundland with a high incidence of the organism as far as 150 miles off the northeast coast. In general, *C. botulinum* appeared to be absent from the shallower bank areas but present in the deeper waters within and bordering upon the Continental Shelf.

LAYCOCK, R. A., AND A. A. LONGARD. 1972. *Clostridium botulinum* in sediments from the Canadian Atlantic seaboard. J. Fish. Res. Bd. Canada 29: 443–446.

Nous avons détecté la présence des types B, C, et E de *Clostridium botulinum* au large des côtes de la Nouvelle-Ecosse. Alors que nous n'avons trouvé les types B et E qu'à de faibles concentrations sur le plateau de la Nouvelle-Ecosse, des poches de haute concentration du type C ont été découvertes dans la région Halifax–Dartmouth et au large, dans les eaux plus profondes du bassin Emeraude et sur le bord du plateau. Le type E n'a été trouvé qu'au large des côtes de Terre-Neuve et, en grande concentration, jusqu'à 150 milles de la côte nord-est. En général, *C. botulinum* semble absent des régions moins profondes des bancs, mais présent dans les eaux plus profondes des chenaux et des abords du Plateau continental.

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IN recent years a renewed interest in possible botulism hazards from fisheries products has arisen with the use, or proposed use, of preservation methods which, while effectively retarding the growth of the normal psychophilic spoilage flora of the product, rely on adequate refrigeration to prevent outgrowth of pathogens less affected, or unaffected, by the treatment given. Examples of such treatments currently being investigated include the pasteurization of shrimp and crab meat (Lerke and Farber 1971), and the use of low level gamma irradiation for extending the storage life of fresh fillets (Segner and Schmidt 1970). Fundamental to a knowledge of the potential botulism hazard, which may arise from insufficient processing, is a knowledge of the distribution of the causative organism *Clostridium botulinum* in the environment. It has been found, for example (Laycock and Loring 1972), that in the Gulf of St. Lawrence *C. botulinum* is present in high concentrations in some important fishing areas and apparently absent from others. In the present investigation, the occurrence of *C. botulinum* of various types on the eastern Canadian Continental Shelf is reported.

Materials and Methods

Bottom samples were collected from the Continental Shelf off Nova Scotia (Fig. 1) and Newfoundland (Fig. 2) between June and October, 1970, using a gravity corer and a pipe dredge, designed in the laboratory. Samples were transferred to 6-oz Whirl Pak Bags then sealed in larger gas and moisture impermeable bags and held in refrigerated storage until tested.

For detection of *C. botulinum* 2–4 10-g samples of material collected at each station were used, fewer samples being tested when stations were in close proximity.

Procedures used for enrichment and detection of *C. botulinum* were similar to those employed successfully for detection of the organism in an extensive survey of the west coast of North America (Eglund and Poysky *1965a, 1967*). The 10-g test samples were inoculated into 50 cm^3 of the enrichment medium, a modification of Dolman's cooked meat medium (Dolman 1957) containing glucose, peptone, trypticase, and yeast extract (Eklund et al. 1967). The inoculated medium was incubated 5 days at 25 C in an atmosphere of 95% nitrogen

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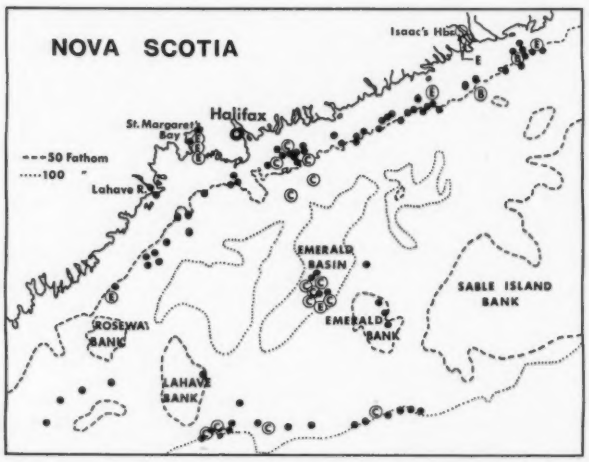

FIG. 1. *Clostridium botulinum* on the Scotian Shelf. Closed circles (●) indicate stations at which *C. botulinum* was not detected. Open circles indicate the presence of *C. botulinum* types B (Ⓑ); C (Ⓒ); and E(Ⓔ).

5% carbon dioxide. Portions (approximately 15 cm³) of incubated medium were centrifuged (13,000 × *g* for 10 min at 3 C) and the presence of toxic material was detected by duplicate mouse intraperitoneal injections of 0.5 ml of supernate diluted 4:1 with gelatin phosphate buffer. Samples lethal to mice within 48 hr were tested for specific toxins of *C. botulinum*. Samples of supernate were diluted 4:1 with gelatin phosphate buffer containing 10,000 mouse anti LD_{50} per cm³ of specific or polyvalent antitoxin. Toxin–antitoxin mixtures were incubated 1 hr at 25 C prior to injection of 0.5 cm³ of each mixture into pairs of mice. Survival of mice protected by a specific antitoxin and death with typical symptoms of all other mice, except those protected by polyvalent antitoxin, was taken to indicate the presence of a particular serotype of *C. botulinum*. No nonspecific toxicities were encountered, but titres of type C toxin were frequently very much higher than those of ot her types present necessitating a 1:10 to 1:100 dilution of samples prior to neutralization to prevent overwhelming of the 1000 anti LD_{50} of antitoxin administered to each mouse.

Trypsin activation of toxin was not employed. Eklund and Poysky (1967) found the majority of their samples positive after trypsinization were also positive in the untrypsinized state. The results of Ward et al. (1967) and Bott et al. (1968) indicated that trypsin activation may destroy rather than potentiate weak positives. The advantages of being able to test a much larger number of samples were considered to outweigh the disadvantages of possibly missing some positive samples in which toxin was present in the inactive form.

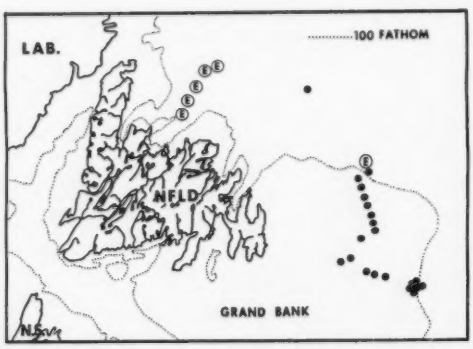

FIG. 2. *Clostridium botulinum* off the coast of Newfoundland. Closed circles (●) indicate stations at which *C. botulinum* was not detected. Open circles indicate the presence of *C. botulinum* type E (Ⓔ).

Results

NOVA SCOTIA

A total of 251 samples from 107 sampling stations were tested for *C. botulinum* toxin. The distribution of the sampling stations and the location of positive samples are shown in Fig. 1.

Estuarine and Bay stations — Samples were taken from three areas along the coast of Nova Scotia. *Clostridium botulinum* type E was found at Isaac's Harbour in 2 of the 12 samples tested, and in 4 of 10 samples from St. Margaret's Bay. None of the 4 samples from the mouth of the LaHave river was positive.

Inshore fishing grounds — Of 121 samples tested from 59 stations in the inshore fishing grounds (up to 25 miles from shore), 12 (10%) were found to contain *C. botulinum*: 2 type B, 7 type C, and 3 type E.

Offshore fishing grounds — A total of 104 samples was tested from 38 stations between Brown's Bank and Sable Island Bank and at distances from shore ranging from 40 miles to the edge of the Continental Shelf (approximately 120 miles). *Clostridium botulinum* was found in 13 samples, 12 type C and one type E.

Newfoundland

Sampling off the east coast of Newfoundland was curtailed due to the inclement weather but 62 samples from 27 stations, predominantly in the Notre Dame Bay and Grand Bank region, were tested. The distribution of sampling stations and the incidence of *C. botulinum* is shown in Fig. 2.

Offshore fishing grounds — 1, Notre Dame Bay area: *Clostridium botulinum* type E was found at all 5 stations sampled between 40 and 150 miles from the mouth of Notre Dame Bay. Overall incidence of the organism in samples was 31%; 2, Grand Bank: None of the 40 samples tested from 19 stations on the Grand Bank was confirmed as containing *C. botulinum* toxin, although there was one unconfirmed mouse toxicity. The incidence of the organism in the area was thus $< 2.5\%$; 3, East coast of Newfoundland: A further 3 offshore stations were sampled between Notre Dame Bay and the Grand Bank. One type E positive sample was obtained just north of the Grand Bank at a depth of 130 fathoms and about 150 miles from shore. Overall incidence from these stations was 17%.

Discussion

The incidence of *C. botulinum* type E on the Continental Shelf off Nova Scotia appears to be low, only 2.5% of inshore samples, and less than 1% of offshore samples was found to contain the organism. In the relatively land enclosed waters of St. Margaret's Bay, however, a much higher concentration of type E (40%), was encountered. This is not unexpected as areas such as bays and lakes, which act as catchment basins for the surrounding land mass, have often been found to be foci of high concentrations of type E (Dolman and Iida 1963).

Of particular interest is the occurrence and distribution of type C off the coast of Nova Scotia. The role of this particular serotype in human botulism has not been irrefutably established, but it is annually responsible for the death of many thousands of wild fowl in the southwestern United States (Jensen and Gritman 1967). The organic sediments of the shallow, stagnant marshes, which commonly comprise wild fowl breeding grounds, are considered a natural habitat of type C. All of the inshore type C-positive samples were found to be concentrated in an area of a little over 300 sq miles just to the east of Halifax, in which 25% of samples was found to contain the organism. Patterns of sediment deposition and bottom currents in this area are not known; however, the absence of type C from other inshore samples, although most were predominantly composed of fine sediment which is indicative of deposition, indicates that type C was derived from the adjacent coastline, which is in fact marshland rich in wild fowl. Whereas *C. botulinum* type E has generally been found to predominate in northern waters, types A and C have been found to predominate in tropical Latin American waters (Ward 1970). It is not inconceivable that local concentrations of type C, such as that observed, have resulted from transference of the organism by migrating wild fowl. That large-scale die-offs of waterfowl in this area have not been reported is possibly because summer temperatures do not remain high enough for the length of time necessary for sufficient toxin to be formed.

The Continental Shelf off Nova Scotia is approximately 100 miles broad, and contains, in addition to a number of banks where water depth is less than 50 fathoms, large depressions in which water depth is greater than 100 fathoms. That these depressions are acting as offshore catchment basins for terrigenous material is indicated by the fact that 23% of samples taken from one of them (Emerald Basin) was found to contain *C. botulinum* type C. In addition, one type E positive sample was confirmed. It is possible that type E, or other *C. botulinum* toxins, may have been present but remained undetected in samples found to contain very high titres of type C toxin. Insufficient antitoxin was available to allow the neutralization of undiluted type C toxin, whereas type E titres were invariably so low that a tenfold dilution would result in a complete loss of toxicity. None of 34 samples taken from surrounding bank areas were found to contain *C. botulinum*. However, farther offshore in deeper waters (around 100 fathoms) along the continental slope, more positive samples were found, and 4 out of 40 samples (10%) were confirmed as containing type C toxin.

Despite numerous releases of drifters, no information has been obtained on bottom drift in the areas between LaHave and Emerald Banks and the coast (Lauzier 1967). The distribution of type C indicates a generally southerly flow of water and material across the shelf in this region, with deposition occurring at least in the deeper waters.

Off the coast of Newfoundland only *C. botulinum* type E was found. Much of the drainage of Newfoundland occurs through rivers running in a northeasterly direction across the province and discharging into the Atlantic through a series of deep incursions into the Continental Shelf, one of the largest of which is that of Notre Dame Bay. All but one of the positive samples were found off this area. Even in samples taken 180 miles from land terrigenous pelites (sediment less than 0.2 μm) predominated, and it is likely that type E could be found considerably farther afield than was tested. The Labrador current flowing southwards over the area provides a further mechanism of transportation. The presence of a positive sample on the northern slope of the Grand Bank, along which a component of the Labrador current flows, possibly is evidence that the current is active in the transport of the organism. The composition of samples taken from the Grand Bank indicated little, if any, sedimentation to be taking place so that the absence of positive samples from this area was not surprising.

Clostridium botulinum can be regarded as a pollutant of the marine environment, and not only has it been found that this organism can be carried considerable distances from land by currents, but also that it can be reconcentrated by deposition in certain offshore areas such as the basins in the Scotian Shelf. Positive samples have also been found far from land, off the Continental Shelf, on the west coast of North America. Type F has been found 52 and 62 miles from shore (Eklund and Poysky 1965a) and type E up to 80 miles from shore (Eklund and Poysky 1965b). These same areas may be expected to similarly concentrate other pollutants associated with the fine sediment fraction, such as pesticides. Emerald Basin, for example, could well be a natural dumpsite for such pollutants discharged into the sea in the Halifax–Dartmouth area.

Because of the diversity observed in the distribution of *C. botulinum* the extent of its occurrence in fish and shellfish can also be expected to vary markedly. Offshore, hake and redfish, for example, are caught in deeper waters such as Emerald Basin, where high concentrations of *C. botulinum* were found, whereas haddock and cod are caught mainly in the shallower Bank areas where *C. botulinum* was not detected. Inshore, local deposition conditions may be expected to determine the extent to which fish and shellfish caught in different areas are contaminated with the organism.

Acknowledgments

We would like to thank Mr T. K. Pitt and the crews of the Fisheries Research Board of Canada vessels, *A. T. Cameron* and *E. E. Prince*, for their help in the collection of the samples; and the National Communicable Disease Center, Atlanta, Ga., for generously providing us with specific botulinal antitoxins unavailable commercially.

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NOTES

Mating Behavior in the Spider Crab, *Chionoecetes opilio*

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WATSON, J. 1972. Mating behavior in the spider crab, *Chionoecetes opilio*. J. Fish. Res. Bd. Canada 29: 447–449.

During the week-long precopulatory embrace, the male spider crab, *Chionoecetes opilio*, held the female, even when attacking intruding males. Before a 45-min copulation period the male assisted female ecdysis. During the postcopulatory embrace spermatophores flowed onto the pleopods of the female. Polygamous males as small as 60 mm width mated with larger females. More than one fertile egg batch was frequently produced from one mating. Females mated successfully up to 95 hr after molting. Hard-shelled females seemed incapable of mating. The probable mating season is February through April and continued recruitment seems assured.

WATSON, J. 1972. Mating behavior in the spider crab, *Chionoecetes opilio*. J. Fish. Res. Bd. Canada 29: 447–449.

Au cours de l'étreinte pré-copulatoire qui dure une semaine, le crabe des neiges mâle, *Chionoecetes opilio*, retient la femelle, même lorsqu'il attaque les mâles faisant intrusion. Avant la copulation qui dure 45 min, le mâle aide la femelle à muer. Durant l'étreinte post-copulatoire, les spermatophores coulent vers les pléopodes de la femelle. Des mâles polygames aussi petits que 60 mm de largeur de carapace s'accouplent avec des femelles plus grandes qu'eux. Un seul accouplement produit souvent plus d'un lot d'oeufs féconds. Les femelles s'accouplent avec succès jusqu'à 95 h après la mue. Les femelles à carapace dure semblent incapables de s'accoupler. La saison probable d'accouplement va de février à la fin d'avril et un recrutement continu semble assuré.

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IN an earlier paper (Watson 1970), mating and egg laying in the spider crab, *Chionoecetes opilio*, were briefly described. This report discusses mating in detail and includes data on polygamy and mating sizes of males.

As females are all less than the commercially acceptable size of 4-inch (102-mm) carapace width, only males are fished. For effective fisheries management, it is especially important to determine the reproductive capabilities of this species and its ability to procreate under such selective fishing conditions.

Materials and methods — Crabs were captured in the Gaspé area of the Gulf of St. Lawrence during October 1969 and May 1970, transported to the laboratory in refrigerated containers, and maintained under natural photoperiod at less than 5 C in compartmented 90×90×30-cm fiberglass tanks (Watson 1970).

Three immature females, likely to molt to maturity,[1] were each transferred to acrylic plastic aquaria 60×60×30 cm. A hard-shelled male was placed with each female so that all phases of mating behavior could be observed. Sixteen other females likely to molt to maturity were placed four to a 45×45-cm compartment in a 90×90×30-cm fiberglass tank, with one hard-shelled male to each group, to see if males were capable of repeated matings. Photographs of mating behavior were supplemented with tape-recorded observations.

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[2]Canadian Contribution to the International Biological Program No. CCIBP-122.

[1]As 50% of females matured at 47-mm carapace width (Watson 1970), immature females above 47 mm were considered likely to molt to maturity.

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Hard-shelled females with late stage eggs were transferred to individual plastic tanks 70×60×60 cm. If new eggs were not produced within 3 weeks of larval release, a male was introduced to see if mating would occur. Mature females caught at sea in May 1970, and about to release larvae, were returned to the sea floor in fine mesh boxes, inside a 1.5×1.5×0.6-m steel-framed crab trap with closed entrances and a cover of 75-mm mesh netting. The trap was inspected daily to see if new eggs were produced in the absence of males.

Results — The complete mating process was observed and documented for two pairs. Several other pairs were seen at various mating stages but detailed observations were not made. As the process was similar in all cases the following description of mating is based on one pair: a hard-shelled 83.9-mm male, weighing 207 g, and a female which after the molt increased from 63.0- to 72.5-mm carapace width and 50- to 80-g live weight.

From November 1969 through January 1970, no interactions between male and female were observed. On February 3, the male grasped the female, which resisted even when brought into the mating position, and the male vigorously flexed his abdomen. During this time the female was not released and was periodically carried about 23 cm from the aquarium floor. On three occasions, another hard-shelled male was introduced, but was soon driven away by the original male who retained his grasp on the female.

At 1430 hr on February 9, the pericardial sacs of the female began to swell and lift up the posterior carapace. The male periodically stroked the carapace near the pericardial sacs with a cheliped and the female became relatively quiet. For the next 5 hr the male continued to stroke the female, but at times quite roughly carried her round the aquarium. At 1940 hr the new carapace was visible (Fig. 1A). As the old shell lifted, the male continued to grasp the female with one chela and stroked or pushed the new carapace with the other (Fig. 1B). When the abdomen began to emerge, the male changed position, held the anterior of the old shell between the proximal portions of his chelipeds and assisted the female out of the old shell (Fig. 1C, D). At 0100 hr February 10, the molt was completed. The male did not release his grasp but ceased to treat the female roughly and held her away from him while standing over the cast shell. No attempt was made to push the old shell away. Within 15 min the female's new shell had swollen to a normal shape.

At this point both crabs began to flex abdomens and vigorously move their mouthparts. At 0130 hr copulation as described by Watson (1970) began, and lasted until 0215 hr (Fig. 1E).

During a postcopulatory embrace the male carried the female away from and in front of him, or allowed her to rest on the aquarium floor. Periodically, both flexed abdomens and spermatophores issued from the male's pleopods. The female's pleopods wafted back and forth drawing spermatophores towards her abdomen (Fig. 1F). This embrace ended at 1020 hr when the male released the female. Egg laying began at 1310 hr.

Males, each of which had been placed with four immature females, were removed in late January 1971 as their rough treatment of some of the females in precopulatory embrace resulted in heavy loss of female limbs and death in four instances. Males were introduced either just before or just after the female molt. Only seven molted to maturity; five remained immature.

Table 1 summarizes the mating observations. One 98.2-mm male mated successfully with six females from February 7 to March 5, 1971. Two matings occurred within a 1-hr period. The smallest male (60.8 mm) in the acrylic plastic aquaria successfully mated with a female which, after molting, increased from 63.9- to 73.7-mm carapace width.

After releasing larvae in the laboratory during April 1970, two old-shell females failed to produce eggs when isolated from males. Attempts to induce mating and egg laying by introducing mature males failed and the experiment was terminated in May 1971. All other females (16 in the laboratory and two in the field) produced fertile eggs after releasing larvae without subsequent contact with males. Eggs were generally laid within 2 weeks of larval release. Females, newly molted to maturity, generally laid eggs within 24 hr after mating had ceased. In only two cases was the start of egg laying observed (Table 1).

Discussion — These mating observations resemble those of Snow and Neilsen (1966) for the Dungeness crab, *Cancer magister*, Edwards (1966) for the European edible crab, *Cancer pagurus*, and Churchill (1921) for the blue crab, *Callinectes sapidus*. The most notable difference was in the precopulatory position. In the above-mentioned species both sexes faced in the same direction and the old shell of the molting female was pushed away by the male. In *C. opilio*, both sexes faced each other, and the male, while holding the old shell between the bases of its chelipeds, pushed the emerging female out. As in *C. magister* and *C. pagurus*, the precopulatory embrace in *C. opilio* lasted about 8 days and the male did not allow other males near the female.

Ecdysis in the female was similar to that described for male *C. opilio* (Watson 1971). The postcopulatory behavior cannot be easily explained. Postcopulatory embraces between old-shell males and new-shell females were observed by Snow and Neilsen

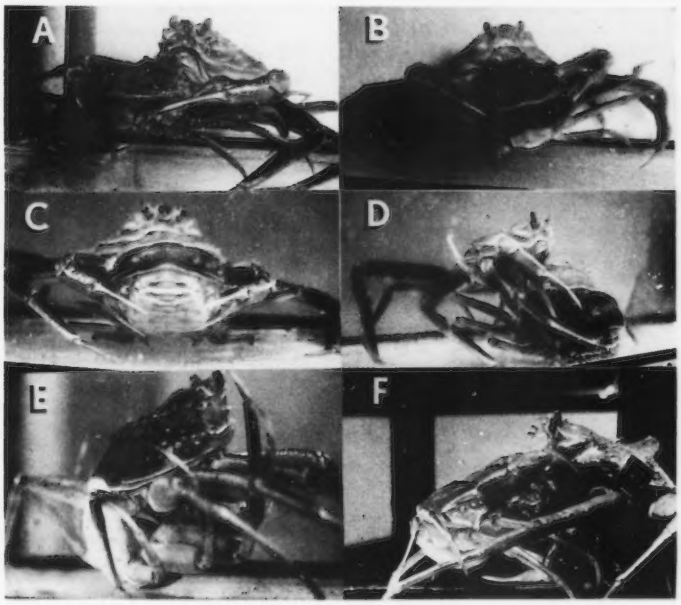

FIG. 1. Reproductive behavior of *C. opilio*. Elapsed times are from start of female ecdysis. A) +30 min, female's new shell appears; B) +4 hr, 30 min, male stroking female with chela; C) +5 hr, 12 min, male assisting female out of shell; D) +5 hr, 19 min, male actively pushing female from old shell; E) +6 hr, copulation; F) +8 hr, postcopulatory embrace, spermatophores issuing from male pleopods.

Watson — J. Fish. Res. Bd. Canada

TABLE 1. Summary of laboratory mating observations on *C. opilio*.

Carapace width (*mm*)			Precopulatory embrace (*days*)	Date maturity molt	Interval (*hr*)	
	Female					
Male	Premolt	Postmolt			Molt to mating	Mating to egg laying
71.2	54.5	63.1	8	8.2.70	0.5	3.5
83.9	63.0	72.5	7	10.2.70	0.75	13.0
60.8	63.9	73.7	9	15.2.70	3.25	<13[a]
98.2[b]	52.3	59.2	–	10.2.71	1.0	<22
	48.2	55.0	–	11.2.71	0.5	< 9
	46.6	52.9	–	24.2.71	–	<23
	48.7	55.3	–	5.3.71	–	<72
(c)	53.0	60.1	–	7.2.71	27	<12
(c)	52.7	60.2	–	6.2.71	53	<11
91.3	52.0	60.3	–	5.2.71	95	< 9

[a]Denotes time after mating eggs first observed. Egg laying started overnight or on weekend.
[b]This male mated with six females, and two matings (c) occurred within a 1-hr period.

(1966) for *C. magister* and by Edwards (1966) for *C. pagurus* but frequent flexing of the abdomen by both sexes, emission of spermatophores by the male, and gathering of spermatophores by the female were not reported. It is possible that this behavior in *C. opilio*, observed in six different mating pairs, may induce egg laying. Eggs were deposited soon after or during this period and many spermatophores were seen within the egg masses. Alternatively, the first batch of eggs may be externally fertilized and subsequent batches may be fertilized from spermatophores stored in the spermathecae.

Concern has been expressed by fishermen and crab processors that, as no breeding pairs have been caught in commercial traps, males may not be attracted to bait while engaged in prolonged reproductive activities. A more credible explanation for the absence of breeding pairs is that mating takes place before the fishery begins in early May. Indeed, Kato et al. (1956) caught grasping pairs of *C. opilio elongatus* in tangle nets in the Sea of Japan from February through April and G. C. Powell (personal communication) captured grasping pairs of *C. bairdi* in Alaskan waters by Scuba diving, only from February through early May. In the laboratory, molts to maturity for females occurred in February or March 3 years in succession. Ice cover prevents sampling and fishing from January through April in the Gulf of St. Lawrence, therefore exact determination of the mating season is difficult. If mating does occur when ice cover prevents fishing, there is, in effect, a natural closed season that will act as a conservation measure in allowing mating to proceed undisturbed.

Mating in the hard-shelled condition, as reported by Hartnoll (1969) for certain brachyuran crabs, has yet to be demonstrated for *C. opilio*. However, as most females, which released larvae both in the field and the laboratory, produced fertile eggs in the absence of males, and as few nonovigerous mature females have been found in field samples (J. Watson unpublished data), it seems that such mating is rare or nonexistent.

The probable mating season, the lack of barren females, and the large number of eggs produced by each female (Watson 1969), indicate that reproductive potential in the population is high and that continued recruitment is assured. In addition, previous concern (Watson 1970) for the effect on reproductive potential of a reduction in commercial size coupled with fishing exclusively for males is somewhat allayed by the fact that males are polygamous and can mate at a size (60-mm carapace width) close to that of maturity (Watson 1970).

However, in areas of heavy exploitation it is possible that males could be reduced to such low densities that some females might not be fertilized for a considerable period after molt to maturity. Templeman (1936) indicated that female lobsters (*Homarus americanus*) might not be fertilized if mating were delayed more than 12 days after the molt. Limited supply of newly matured females precluded extensive experiments on *C. opilio*. Three females mated successfully 27, 53, and 95 hr after molting (Table 1). Further research is required to determine the maximum time after female molt that mating can be successful.

Acknowledgments—Thanks are due to P. G. Wells and R. M. Ireland who helped observe and record the mating process, and to Drs J. F. Caddy and J. S. Scott, who critically reviewed the manuscript. Funding for this study was provided in part by the Industrial Development Branch, Fisheries Service, Department of Environment, Canada. Illustrations were prepared by P. W. G. McMullon.

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Distribution of Iron, Magnesium, Copper, Zinc, and Silver in Oysters Along the Georgia Coast

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WINDOM, H. L., AND R. G. SMITH. 1972. Distribution of iron, magnesium, copper, zinc, and silver in oysters along the Georgia coast. J. Fish. Res. Bd. Canada 29: 450–452.

The shells and soft tissues of approximately 50 oysters (*Crassostrea virginica* Gmelin) collected along the Georgia coast were analyzed for iron, magnesium, copper, zinc, and silver. Magnesium was the only metal found to be more concentrated in the shells. Correlations between the metals in the soft tissues suggest that iron and magnesium, and copper, zinc, and silver form two separate groups of metals which are concentrated similarly by the oyster. Geochemical characteristics of a given metal are an important control in their uptake by the oyster.

WINDOM, H. L., AND R. G. SMITH. 1972. Distribution of iron, magnesium, copper, zinc, and silver in oysters along the Georgia coast. J. Fish. Res. Bd. Canada 29: 450–452.

Les auteurs ont effectué des analyses de fer, magnésium, cuivre, zinc, et argent sur l'écaille et les tissus mous d'environ 50 huîtres (*Crassostrea virginica* Gmelin) récoltées le long de la côte de la Georgie. Le Mn est le seul métal présent en plus grande concentration dans l'écaille que dans les tissus. La corrélation entre les métaux des tissus mous suggère que le fer et magnésium d'une part, et le cuivre, zinc, et argent de l'autre, sont deux groupes de métaux concentrés de la même façon par l'huître. Les caractéristiques géochimiques d'un métal donné sont un facteur important de contrôle de sa captation par l'huître.

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MARINE organisms, especially bivalve molluscs, are able to concentrate heavy metals from the environment. This note reports the results of a survey of the distribution of iron, manganese, copper, zinc, and silver in oysters (*Crassostrea virginica* Gmelin) collected at locations representing different environments along the coast of Georgia.

Methods — Oysters were collected from 10 stations of various salinity ranges along the Georgia coast. The oysters were shucked while still alive and the soft parts were drained. All detritus was cleaned from exterior surfaces of the shells, which were dissolved in hydrochloric acid. The resulting solution was analyzed by atomic absorption spectrophotometry, using standards and blanks in a reagent grade calcium carbonate matrix. The concentration of iron, magnesium, copper, zinc, and silver in the reagent $CaCO_3$ was below detectability with the analytical methods used.

The soft parts of the oysters were dried overnight at 105 C, their moisture content ranging between 83–87%. The samples were then ashed at 600 C, broken down further with hydrofluoric acid and the residue was dissolved in nitric acid. Analysis of the resulting solutions was by atomic absorption spectrophotometry, using standards in a matrix containing sodium, calcium, magnesium, and potassium in approximately the same concentrations as found in the sample solutions.

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Precision of the analysis for zinc in the oysters is about ±5% of the stated value. The precision for the other metals is better than ±5%. In the shells all analyses have a precision of about ±5%.

Results and discussion — Table 1 gives the means and standard deviations of the heavy metal analyses in the shells and soft parts of oysters from the various locations. Copper and silver were below detectability in the shells (less than 1 ppm). The results of shell analysis show no correlation with those of soft tissues. Lesser concentrations of all metals analyzed occurred in the shell than in the soft tissues, excepting manganese which was considerably higher in the shells.

To consider the processes involved in the uptake of the five metals by oysters in relation to their geochemical behavior, each was correlated with the other as shown:

Mn	Cu	Zn	Ag	
0.67	–0.12	–0.13	–0.05	Fe
	–0.15	–0.08	0.12	Mn
		0.79	0.56	Cu
			0.66	Zn

Significant correlations (underlined) have coefficients greater than 0.5. Silver, copper, and zinc correlate

TABLE 1. Mean concentration and standard deviation of metals in oyster samples (Concentration on dry weight basis).

Station no.	Salinity ‰	Shells				Soft tissue					
		No. of samples	Fe (*ppm*)	Mn (*ppm*)	Zn (*ppm*)	No. of samples	Fe (*ppm*)	Mn (*ppm*)	Cu (*ppm*)	Zn (%)	Ag (*ppm*)
1	19–32	5	38±30	121±36	3.0±1.4	6	405±63	35±10	261±105	0.57±.22	–[a]
2	20–31	3	31±31	52±30	2.6±0.5	3	528±139	43±4	87±18	0.16±.08	–
3	0–24	5	17±11	33±3	2.2±0.6	6	738±266	40±11	75±35	0.23±.10	–
4	11–25	5	27±16	42±11	1.9±1.2	6	555±189	51±15	62±25	0.21±.09	–
5	5–33	5	12±4	62±28	2.2±1.2	6	372±107	35±11	48±23	0.12±.05	28±16
6	3–22	5	14±8	45±9	1.5±0.9	6	275±77	24±9	112±46	0.22±.04	42±10
7	0–18	5	17±5	42±9	4.6±2.4	6	356±57	33±11	114±26	0.36±.12	82±13
8	15–30	5	15±11	45±12	2.7±1.9	5	233±40	24±6	91±19	0.35±.07	57±20
9	20–33	5	79±60	70±22	8.1±4.5	5	357±165	32±14	68±17	0.15±.02	34±10
10	15–31	5	34±14	36±10	5.9±1.7	5	399±241	30±14	55±22	0.21±.05	36±10

[a]Analysis for silver was not attempted on samples from stations 1 through 4.

significantly with each other but not with iron and manganese. On the other hand, iron and manganese correlate significantly with each other. It therefore appears that the five metals in this study can be broken into two groups.

If it is assumed that the concentrations of the metals in the oysters are dependent primarily on the form of the metal (colloidal, labile and non-labile complexes, free ion) at the point of uptake by the oyster, the two groups of metals probably represent two different forms, that of iron and manganese being distinctly different from that of copper, zinc, and silver.

If the concentration of the five metals studied involves the uptake of the free metal ion only, then all should be concentrated in a similar way, with the exceptions of silver and iron. Manganese, copper, and zinc all occur in solution under normal seawater conditions, mostly in the + 2 valence state as dissociated ions or labile complexes, while silver and iron occur in the + 1 and + 3 valence states, respectively. The divalent ions all have approximately the same ionic radius, and the stabilities of their organic complexes are apparently similar, with copper > zinc > manganese (Goldberg 1957). If these metals exist as simple ions or labile complexes in the + 2 valence state, there should be a correlation between their concentration in the oysters, assuming that uptake involves complex formation with mucus lining of physiologically important organic ligands. Since this is not the case, it would appear that the five elements studied fall into two groups, because they exist in the oyster's environment in distinctly different forms. For example, iron and manganese are brought to the estuarine environment from fresh water runoff in relatively high concentrations. Upon reaching the estuarine environment, the increased *p*H causes their conversion to hydrated oxides, which probably exist as colloids and remain in suspension for some time so that Mn and Fe in the water column exist primarily as hydrated oxides (Windom et al. 1971; Windom and Beck 1971; Morgan and Stumm 1964). Iron (and probably manganese) in colloidal form is readily absorbed by oysters (Galtsoff 1964, p. 388). Copper, zinc, and silver, on the other hand, have concentrations in freshwater runoff similar to their concentration in estuarine waters (Windom et al. 1971; Turekian et al. 1967), suggesting that they occur in estuarine waters as simple ions or as labile complexes with inorganic ligands.

Geochemically, silver, copper, and zinc are more similar to each other than to iron and manganese, and the latter are geochemically more similar to each other than to the former three. The above data therefore indicate that the concentration of metals by oysters is possibly more affected by the geochemistry of the metals than by their biochemistry.

Acknowledgments — We are indebted to D. Menzel and R. Stickney for critically reading the manuscript of this paper. We wish to thank R. Reimold and C. Durant of the University of Georgia Marine Institute at Sapelo Island, Ga., for collecting the oyster samples for this study. Mr F. Bousquet was of great assistance in the analysis of the samples.

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Aging Round Whitefish (*Prosopium cylindraceum*) of the Leaf River, Ungava, Quebec, by Otoliths

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JESSOP, B. M. 1972. Aging round whitefish (*Prosopium cylindraceum*) of the Leaf River, Ungava, Quebec, by otoliths. J. Fish. Res. Bd. Canada 29: 452–454.

Ages assigned to 503 round whitefish (*Prosopium cylindraceum* (Pallas)) from the Leaf River on the basis of otoliths differed notably from those assigned on the basis of scales only for fish above age 8. The hyaline zones in otoliths corresponded to the scale annuli, and the growth increments in otoliths at various dates as percentages of the totals during the collection period were similar to those for scales. Otolith radius increased linearly with fork length, as did otolith weight with fish age.

JESSOP, B. M. 1972. Aging round whitefish (*Prosopium cylindraceum*) of the Leaf River, Ungava, Quebec, by otoliths. J. Fish. Res. Bd. Canada 29: 452–454.

L'âge otolithaire de 503 ménomini ronds (*Prosopium cylindraceum* (Pallas)) provenant de la rivière aux Feuilles ne diffère notablement de l'âge scalaire que chez les poissons âgés de plus de 8 ans. Les zones hyalines de l'otolithe correspondent aux annuli de l'écaille. L'augmentation de taille des otolithes à diverses dates, exprimée en pourcentage de la croissance totale durant toute la période d'échantillonnage, est la même que celle des écailles. Le rayon de l'otolithe augmente linéairement par rapport à la longueur à la fourche. Il en est de même du poids de l'otolithe par rapport à l'âge du poisson.

Received August 18, 1971

THIS report compares ages read from scales and otoliths of round whitefish, *Prosopium cylindraceum* (Pallas), from the Leaf River, Ungava, and presents indirect evidence for the validity of using otoliths to age this species. Scales are an accepted method of aging round whitefish (Bailey 1963; Mraz 1964; MacKay and Power 1968; Normandeau 1969). To my knowledge, no information is available in the literature on the use of otoliths to age any coregonid species. However, otolith–scale comparisons have been made for several salmonids (Nordeng 1961; Grande 1964; Bilton and Jenkinson 1968).

Both scales and otoliths were collected from 508 round whitefish from the Leaf River between June 28 and September 8, 1968. Most fish were caught in 0.5–2.5- inch stretch mesh monofilament gillnets, and the remainder by beach seine. Fork lengths ranged from 110 to 365 mm.

Scale interpretation — From 5 to 10 scales were removed from the left side of each fish, immediately posterior to the termination of the dorsal fin and 2–3 rows above the lateral line. They were mounted on plastic slides (Power 1964) and later examined, without further preparation, at magnifications to 80×. All scale samples were independently read twice by the author (with an agreement of 91.6%), then a third reading was made of only those samples in which ages disagreed, and a final age was assigned on the basis of majority agreement. Each sample of scales contained at least one scale suitable for aging, and no samples were rejected as being completely unreadable. The Petersen method (Graham 1929) of constructing a length-frequency histogram was used to assist in recognition of the annuli of fish to age 3, as the number of histogram peaks corresponded to the number of scale and otolith annuli. The large scale size and distinct separation of annuli (Fig. 1) permitted easy aging of fish up to age 9, but in older fish more difficulty was experienced because of crowding of the outer annuli.

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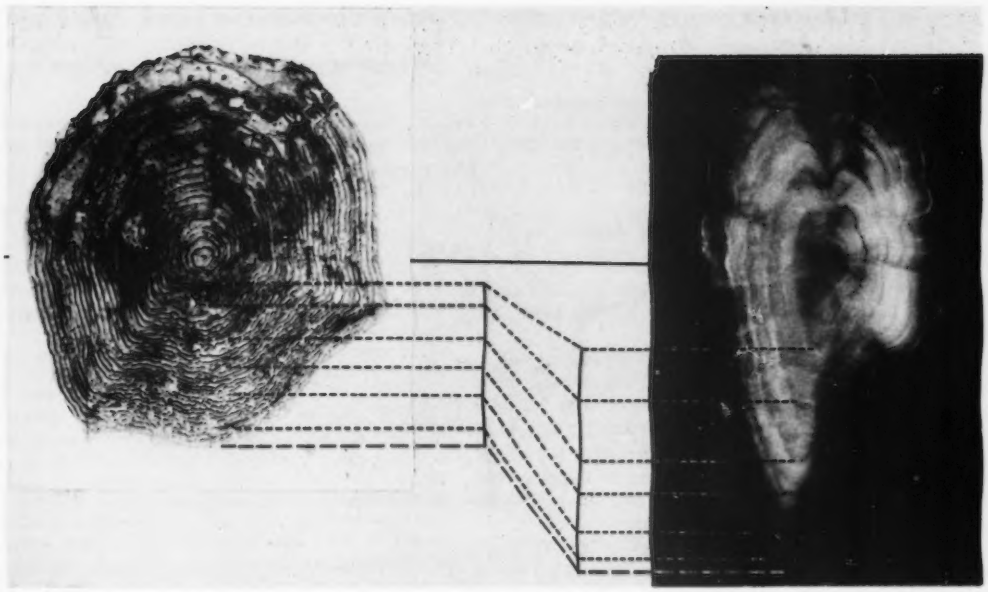

FIG. 1. Annuli of a scale and an otolith from a mature female round whitefish, 260 mm long, age 6+, from the Leaf River. There are equal numbers of zones in scale and otolith, and corresponding variations in summer zone width.

Jessop — J. Fish. Res. Bd. Canada

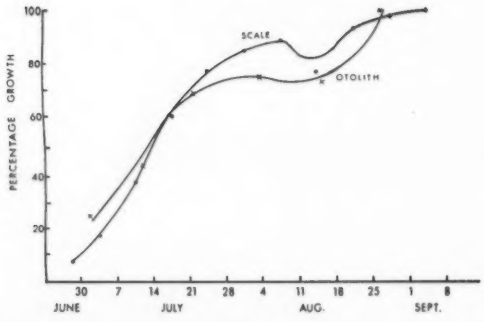

FIG. 2. Mean growth increments as percentages of the total growth between June 28 and September 6 of 45 scale and 37 otolith samples from round whitefish, age 6+, from the Leaf River.

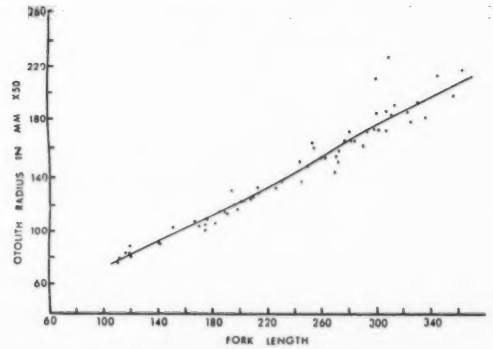

FIG. 3. Relation between otolith radius (*focus–rostrum*) and fork length (*mm*) of 62 round whitefish from the Leaf River. The line was fitted by eye.

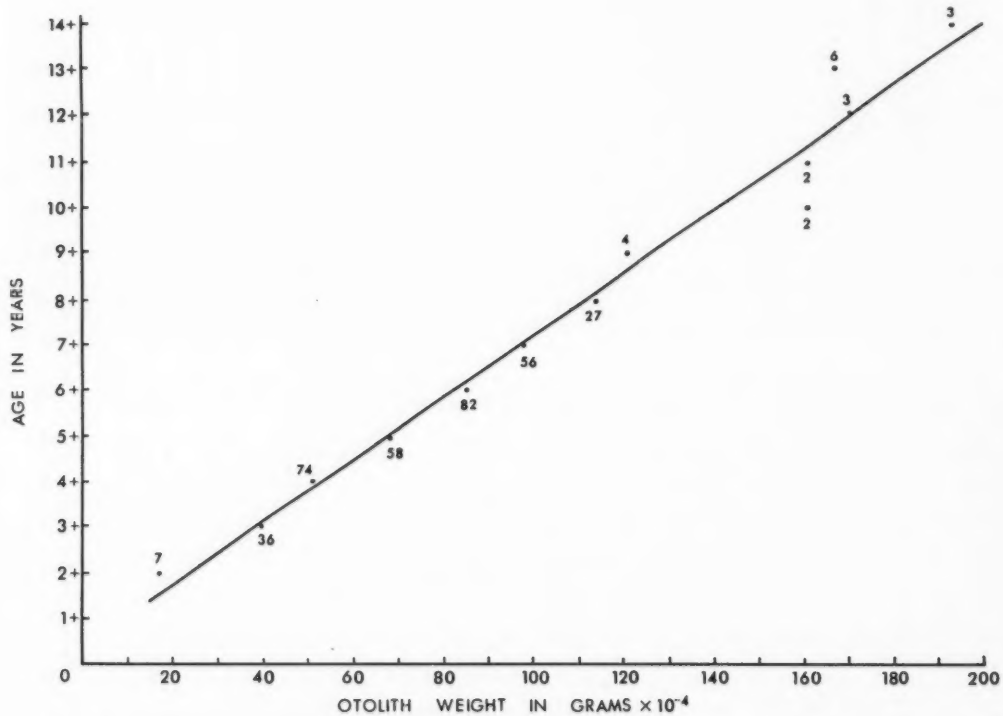

FIG. 4. Relation between mean otolith weight and age of 360 round whitefish from the Leaf River. The sample size accompanies each plotted value. The line was fitted by eye.

Otolith interpretation — For 150 of the fish, cleaned otolith pairs were preserved in glass vials containing a 50% solution of glycerol and water. The method was abandoned as unnecessary and all other otoliths were stored dry. All dry otoliths were weighed to the nearest 10^{-4} g before further use. Following Nordeng (1961), 67 pairs of otoliths were cast in methyl methacrylate to permit easy handling while grinding to expose the otolith zone structure. Before viewing under a stereomicroscope, the ground surface was cleared with creosote. This method was time-consuming and unnecessary. The remaining uncast otoliths were easily read after being soaked in the water-glycerol solution for 24 hr, placed on a matte black background covered with creosote, and illuminated from above with a high intensity lamp. The numbers of otoliths read by the two methods were:

	Uncast	Cast	Total
Readable	439	64	503
Unreadable	2	3	5
Total	441	67	508

The alternating opaque and hyaline zones in otoliths were most easily read on a line from the nucleus to the tip of the rostrum (Fig. 1). The hyaline zone was taken to represent material deposited during the period of slow winter growth, and to be comparable to the annuli on the scales. Age determinations from otoliths were made independently of those from scales. Otoliths were read only once.

Otoliths were readily interpreted because of their relatively large size (1.5–7.0 mm) and distinct zonation (Fig. 1). Narrow, less distinct accessory zones occurred occasionally between major hyaline zones, but created little difficulty in interpretation. The increased blurring of divisions between the outer zones, and the crowding of zones, made it difficult to age older fish. The first one or two hyaline zones also were difficult to distinguish on occasion because of the thickening of the otolith body with age. A small number of otoliths displayed a distinct, narrow hyaline zone between the focus and the first pronounced hyaline zone (Fig. 1). Nordeng (1961) suggested that in Arctic char otoliths, such a zone is formed on transition to free feeding after the absorption of the larval yolk sac. This may be true for the round whitefish.

Otolith usefulness — Estimates of age using scales and otoliths showed a 96.8% agreement (Table 1). Beyond age 8, the percentage of disagreements increased markedly, with the otolith readings generally higher than the scale readings. The difference usually was one year, but occasionally two.

Measurements of scales and otoliths were made at a magnification of 50 ×, and those for samples of age-6 fish were used to estimate mean growth increments as percentages of the total growth of scales and otoliths during the collection period. The collection period (June 28–September 8) represented the major part of the growing season (Jessop MS 1968).

TABLE 1. Percentages of agreement in ages of 503 round whitefish as determined from scales and otoliths.

Age	Total read	Percent agreement
2	8	100.0
3	53	94.3
4	94	98.9
5	74	100.0
6	144	98.6
7	77	98.7
8	29	93.1
9	5	80.0
10	3	100.0
11	5	60.0
12	4	50.0
13	4	100.0
14	3	33.3
Total	503	96.8

The initiation of growth and rate of growth in scales and otoliths were similar. The proportionate seasonal growth increments (Fig. 2) indicate that these otoliths can be used, as the scales are (Bailey 1963), for studying growth rates. By early July, all fish of age-5 or less showed some summer growth on both scales and otoliths, while a small percentage of fish of age-group 6 and older showed no discernible growth in either. As for the age-6 fish, a decrease in the growth rate in early August was evident in similar graphs constructed for four other age-groups.

A linear relation was found between otolith radius and fork length, (Fig. 3) and between mean dry weight of otoliths and fish age (Fig. 4) in representative samples of 62 and 360 fish, respectively.

Any difficulty in obtaining and utilizing otoliths, as opposed to scales, for aging might be offset by the considerations that malformed or unreadable otoliths are few, and that otoliths might offer a more complete record of the early growth of the fish than do scales. Koo (1962) has noted that otoliths of some salmonids are already formed when fry leave the gravel. Presumably this is true of Leaf River round whitefish, while the scales in the area of scale sampling are formed at a fry length of 45–50 mm (Jessop MS 1968). The otolith may be of value in the calculation of growth histories for this species.

Discussion — The close agreement between scale and otolith age determinations indicates that oto-

liths provide a valid alternative to scales for aging round whitefish from the Leaf River, and are possibly more accurate for aging older fish. That ages on the basis of scales and otoliths differed notably only in the older age-groups has been observed in several species (Kohler and Clark 1958; Nordeng 1961; Messieh and Tibbo 1970).

The large size of the scales and otoliths, and the slow growth rate of this species in the subarctic (MacKay and Power 1968), undoubtedly contributed to the ease of reading these structures. Nordeng (1961) found a relation between the clarity of the hyaline zone and growth rate in the Arctic char, so that the slower the growth the more distinct the zones. Crowding of the outer annuli was less severe in otoliths than in scales, although Messieh and Tibbo (1970) noted the opposite in herring scales and otoliths.

The opaque zone was formed during the period of maximum otolith growth, a finding reported by Nordeng (1961) and Koo (1962) for several salmonids. A plot of age versus otolith weight produced a linear relation similar to that obtained by Trout (1954) for Barents Sea cod. The evidence presented indicates that there is a relation between otolith ring growth and body growth, and that one opaque and one hyaline zone are added yearly to the otolith of round whitefish from the Leaf River. Trout (1954), Irie (1960), and others have observed such relations in several fish species.

The decreased growth rate of fish in August (Fig. 2) may have been related to a sudden decrease in water temperature that occurred between the 1st and 3rd weeks of the month. The limited sample sizes taken on the dates involved might also be a factor.

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Marking Larval Largemouth Bass with Radiostrontium[1]

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CARLSON, C. A., AND M. H. SHEALY, JR. 1972. Marking larval largemouth bass with radiostrontium. J. Fish. Res. Bd. Canada 29: 455–458.

Largemouth bass (*Micropterus salmoides*) were marked by exposing newly-hatched prolarvae to 6.4, 11.3, and 16.8 μCi ^{85}Sr/liter pond water for 7 days. Embryos were collected by pipette from nests in ponds and incubated to hatching in disposable aquaria in the laboratory. Radiostrontium accumulation from water continued throughout the first week of postembryonic life but was most rapid after the fourth day of exposure. In another study the techniques were found useful for quantifying predation on larval bass by aquatic invertebrates.

CARLSON, C. A., AND M. H. SHEALY, JR. 1972. Marking larval largemouth bass with radiostrontium. J. Fish. Res. Bd. Canada 29: 455–458.

Nous avons marqué des achigans à grande bouche (*Micropterus salmoides*) en exposant les poissons nouvellement éclos à des teneurs de 6.4, 11.3 et 16.8 μCi de ^{85}Sr/litre d'eau d'étang durant 7 jours. Les embryons ont été prélevés à la pipette sur les nids des étangs et ont été incubés jusqu'à l'éclosion dans des viviers remplaçables au laboratoire. L'accumulation de radiostrontium s'est poursuivie durant la première semaine de vie post-embryonnaire, mais elle était plus rapide après le quatrième jour d'exposition. Cette technique a été utile dans une autre expérience pour quantifier la prédation des invertébrés aquatiques sur les larves d'achigan.

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THIS report describes techniques for marking larval largemouth bass (*Micropterus salmoides*) with radiostrontium. The methods described were found to be useful in marking young bass for evaluation of predation by aquatic invertebrates (Shealy and Carlson 1972) and may be useful in marking larval fishes for other purposes. We selected ^{85}Sr for this study because it emits gamma rays (0.51 Mev), which allow detection and measurement in living bass larvae and their predators. Its physical half-life (64 days) ensures persistence throughout larval development. Strontium is readily soluble, and it can be incorporated into fish tissues either by absorption from food or by direct absorption from surrounding water (Auerbach et al. 1971; Ophel and Judd 1967, 1969).

Procedure — Larval bass used in this study were produced in ponds at the Cornell University Fishery Laboratory. Ponds were checked frequently for reproductive activity once the spawning period began. When a new nest was discovered, fertilized eggs were collected with a 25- or 50-ml pipette (with 3- to 4-mm opening) and placed in plastic aquaria with water from the nest site. Frequency of pond inspections was such that time of fertilization (and hence age of embryos) was usually known within 4–6 hr. Additional pond water was collected within a few meters of the nest and filtered to exclude macroorganisms and coarse particulate material.

In the laboratory, 2.5 liters of this water and then approximately 250 embryos were placed in each of 15 6-liter plastic aquaria. All aquaria were aerated (two airstones each), and water temperatures were held between 20–23 C throughout the experiments. Dead embryos were removed daily with stovepipe forceps with wire-loop extensions. Only aquarium lots in which the hatch exceeded 95% were used. Marking was done in the same aquaria in which larvae hatched to avoid handling of sensitive prolarvae. All larvae in a given experiment were of the same age, but since more than one female may spawn in a single nest, we could not be certain that all had identical parentage.

Experimental aquaria were located on metal shelves covered with Kimpak "diaper" paper (absorbent on the upper surface and water-repellent on the bottom). Each shelf held three 50×61×8-cm (deep) plastic photographer's trays lined with the same paper. Two 20-liter glass aquaria were placed in each tray, and a plastic experimental aquarium (6 liters) was placed inside each glass aquarium. Outer aquaria and the trays were provided primarily to contain accidental spills of radioactive liquid. Plexiglass lids for inner and outer

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aquaria (with holes for 5-mm ID air tubes) minimized spattering and evaporation of inner aquarium contents. Tap water was added to each outer aquarium to stabilize temperatures and reduce exposure of the experimenters to radiation. Larvae were exposed to 10–12 ft-candles incident light during the lighted period (0800–2400 hr) each day except during sampling periods, when they were exposed to 50–75 ft-candles.

When embryos had hatched, ^{85}Sr was added to nine of 15 inner aquaria: about 15, 30, and 45 μCi each to three. Three inner aquaria (controls) received no radiostrontium, and three others were used only for water chemistry determinations. After addition of ^{85}Sr, aeration was resumed to provide mixing.

Every 24 hr for 7 days, 10 larvae and a 10-ml water sample were removed from each inner aquarium for radioactivity determinations. Water samples were collected to compare concentrations of ^{85}Sr in experimental aquaria and to monitor dispersion. After collection, they were placed in low-background screw-top scintillation vials and labelled for subsequent identification. Larval fish samples, collected by pipette were placed (with a minimum of aquarium water, generally 1 to 2 drops) in 100-ml plastic beakers. Each fish sample was poured from its beaker into a Millipore filtration apparatus containing a 57-mm filter (HAWP, 0.45 μ pore size) and rinsed with distilled water and 0.1 N HCl. The filtration apparatus was then dismantled, and the filter and 10 fish were removed by forceps to a scintillation vial. The filtration apparatus was rinsed with 0.1 N HCl before reassembly to avoid contamination of the next sample. Filters were dried in open vials for 1–2 hr. Finally, 10 ml acetone was pipetted into each vial to dissolve the filter, and the vials were capped and labelled.

Radioactivities of fish and water samples were determined with a Baird-Atomic Model 530 Scintillation Spectrometer in conjunction with a Baird-Atomic Model 810C Well Scintillation Detector. Use of three aquaria at each ^{85}Sr concentration provided three simultaneous replications of each treatment.

We tested various methods of rinsing larval fish in the filtration apparatus to remove surface-adsorbed ^{85}Sr. Rinses with 0.1 N HCl proved more effective than similar rinses with distilled water. Brief immersion of larvae in 0.1 N HCl was extremely effective in lowering residual radioactivity but was not continued because it apparently removed more than surface-adsorbed ^{85}Sr. A standardized series of rinses (separated by suctioning) was eventually adopted: fish were rinsed with 50 ml distilled water, a swirl (about 30 ml) of 0.1 N HCl, and two 10-ml aliquots (measured with a cantipette) of 0.1 N HCl. These rinses effectively removed radioactive aquarium water transferred with fish to the filtration apparatus.

As various chemical conditions may affect the accumulation of radiostrontium by fishes (Brungs 1965; Polikarpov 1966; Townsley 1963), conditions in the aquaria were analyzed by methods prescribed by the American Public Health Association (1966). Chemical conditions remained relatively constant over 1.5 weeks in our experimental aquaria. At 20–23 C, dissolved O_2 ranged from 8.0 to 8.5 mg/liter, free CO_2 from 0.6 to 1.4 mg/liter, *p*H from 7.3 to 7.6, total alkalinity (as $CaCO_3$) from 81 to 92 mg/liter, and EDTA hardness (as $CaCO_3$) from 110 to 168 mg/liter. Atomic absorption spectrographic analysis of the water indicated a stable calcium concentration of 20.0 mg/liter and a stable strontium concentration of 0.04 mg/liter.

Usefulness of the techniques — The techniques described for handling larval bass and marking them with radiostrontium were satisfactory. Bass embryos were transferred to the laboratory and incubated to hatching with better than 95% survival, handling of sensitive late embryonic and early prolarval stages was minimized, and gamma-emitting ^{85}Sr was quickly introduced into the larvae. Mortality during exposure to ^{85}Sr was less than 5%, except in rare instances when all larvae in an aquarium succumbed as fungus spread from small clusters of dead larvae. Larvae showed no apparent ill effects of continuous immersion in ^{85}Sr concentrations up to approximately 17 μCi/liter for a week after hatching.

Results of an experiment on prolarvae hatched from embryos collected on June 12, 1969, illustrate the results and data analysis. Radioactivity determinations of 10-ml water samples indicated that, after 24 hr, mean ^{85}Sr concentrations in the low-, intermediate-, and high-activity aquaria were 6.4, 11.3, and 16.8 μCi/liter (standard deviations of these means were 0.8, 0.1, and 0.3 μCi/liter), respectively. The discrepancy between the ratio of radioactivity of low-, intermediate-, and high-activity aquarium contents (1:1.66:2.45) and the expected ratio (1:2:3) may have resulted from increased adsorption to aquarium walls at higher ^{85}Sr concentrations. It seems unlikely that consistent discrepancies of this magnitude resulted from errors in introduction of radiostrontium or inadequate mixing of the ^{85}Sr solution added to aquaria. Differences in samples collected at a given time from the surface and bottom of an aquarium were usually less than 1% of their mean radioactivity.

The average radioactivity of fish collected from experimental aquaria increased over the 7-day exposure period, though daily variation was considerable (Table 1). Accumulation of ^{85}Sr was more rapid after the fourth day of larval life than before. Fish in the intermediate- and high-activity aquaria had remarkably similar radioactivities through the fifth day of exposure, but those in the high-activity aquaria had the higher values on the sixth and seventh days.

Rinses with 0.1 N HCl in the filtration apparatus often resulted in partial disintegration of the soft tissues (e.g. yolk sacs) of early prolarvae. This disintegration may partially explain the similarity in ^{85}Sr accumulation at the three exposure concentrations

TABLE 1. Mean radioactivities (pCi above background) of 10-fish samples from three replicate aquaria at each ^{85}Sr concentration during 7 days of exposure.

Days of exposure	^{85}Sr Concn (μCi/liter)		
	6.4	11.3	16.8
1	0 (0)[a]	50 (14)	26 (10)
2	42 (10)	62 (8)	61 (8)
3	111 (57)	174 (84)	180 (28)
4	68 (36)	139 (42)	116 (98)
5	128[b]	308 (45)	298 (105)
6	180[b]	444 (123)	531 (159)
7	361[b]	625 (93)	937 (223)

[a]One standard deviation of mean.

[b]Mean of two samples, as mortality prevented collection of 10 living larvae from one of the aquaria.

during the first 4 days of sampling and increasing differences in accumulation between ^{85}Sr concentrations after 5 days of exposure. Early prolarvae may have had much of their radiostrontium body burden removed by rinsing, while older larvae, with better-developed and firmer tissues, may have retained more of their accumulated ^{85}Sr. Increased uptake after 4 or 5 days of prolarval life, especially at higher ^{85}Sr concentrations, may also be explained by commencement of bone- or scale-forming activity. However, the times of onset of bone and scale formation in larval bass have not yet been determined.

After larvae had been exposed to ^{85}Sr for 5 days, additional fish samples were collected to determine the variability in radioactivity of samples of fewer than 10 larval bass. In addition to the usual sample of 10 fish, samples of 1, 2, and 6 fish were removed from each aquarium and rinsed, stored, and "counted" as described above. There was considerable variability between replicate samples at each concentration, but means of the replicates showed a general relationship between radioactivity and number of fish (Table 2). A sample size of 6 to 10 fish is desirable, however, to reduce standard deviations of sample means and increase confidence in estimates.

In a study of retention of the radioactive marks (Shealy and Carlson 1972), prolarval bass immersed for 72 hr in 18, 34, and 48 μCi/liter of water retained 66% of their radioactivity 96 hr after transfer to nonradioactive water. Bass embryos lost their marks far more rapidly, and postlarvae retained their marks much longer than did prolarvae. The biological halflife of ^{85}Sr in postlarval bass was approximately 45 days.

Marks introduced by these techniques were found to be satisfactory for studies of invertebrate predation on largemouth bass through the first summer of the life of the bass. Our techniques appear applicable to other fishes with demersal eggs and short incubation periods (e.g., other centrarchids), and similar techniques might be useful for marking fish embryos.

TABLE 2. Mean radioactivities (pCi above background) of triplicate samples of 1, 2, 6, and 10 larval bass after 5 days exposure to three concentrations of ^{85}Sr.

No. of fish in sample	^{85}Sr Concn (μCi/liter)		
	6.4	11.3	16.8
1	18 (11–30)[a]	33 (19–43)	37 (34–42)
2	31 (13–51)	67 (57–78)	73 (64–82)
6	76 (52–103)	174 (133–204)	186 (159–225)
10	128 (90–166)[b]	308 (262–351)	298 (186–395)

[a]Range.

[b]Mean of two samples, as mortality prevented collection of 10 living larvae from one of the aquaria.

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Effects of the Floy Anchor Tag on the Growth and Survival of Brook Trout (*Salvelinus fontinalis*)

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CARLINE, R. F., AND O. M. BRYNILDSON. 1972. Effects of the Floy anchor tag on the growth and survival of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Bd. Canada 29: 458–460.

We conducted two field trials to determine the effects of the gun-injected, Floy anchor tag (FD-67) on growth and survival of 10-month-old domesticated brook trout (*Salvelinus fontinalis*) in a 0.7 ha pond. Trials ran from October to the following June. In 1968 we stocked 106 tagged trout and 1005 controls, and in 1969, 300 tagged trout and 500 controls. We removed different fins from each group to determine tag loss. In both trials we sampled the trout twice through the ice and twice during open water. The tags retarded growth of the trout from October to January; differences in growth rates between tagged and controls were 18 and 28% for the 1968 and 1969 trials, respectively. After January both groups grew at similar rates. Thetags did not affect survival, and tag losses were 5.7 and 2.0% for the two trials.

CARLINE, R. F., AND O. M. BRYNILDSON. 1972. Effects of the Floy anchor tag on the growth and survival of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Bd. Canada 29: 458–460.

Nous avons fait deux essais sur le terrain en vue de déterminer l'influence de la marque de Floy, injectée au fusil (FD-67), sur la croissance et la survie d'ombles de fontaine (*Salvelinus fontinalis*) élevés en pisciculture, âgés de 10 mois et gardés dans un étang de 0.7 ha. Les essais se sont poursuivis d'octobre à juin de l'année suivante. Nous avons ensemencé 106 poissons marqués et 1005 témoins en 1968 et 300 poissons marqués et 500 témoins en 1969. Nous avons coupé différentes nageoires sur chaque groupe afin d'évaluer les pertes de marques. Au cours de chacun des essais, nous avons prélevé des échantillons d'ombles deux fois à travers la glace et deux fois en eau libre. Les marques ont ralenti la croissance des ombles de fontaine d'octobre à janvier; le rythme de croissance des individus marqués différait de 18 et 28% de ceux des témoins au cours des essais de 1968 et 1969, respectivement. Après janvier, la croissance des deux groupes s'est poursuivie au même rythme. Les marques n'ont pas affecté la survie, et les pertes de marques ont été de 5.7 et 2.0% au cours des deux essais.

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THE purpose of this paper is to report effects of gun-injected Floy FD-67 anchor tags on growth and survival of 10-month-old domesticated brook trout (*Salvelinus fontinalis*). We chose these tags because they are easily and rapidly applied and are small enough to use on yearling trout. Preliminary testing indicated that trout greater than 150 mm in total length could be tagged with little mortality.

Methods and materials — We conducted field trials from October to June in 1968 and 1969. The trout, from Crystal Springs State Hatchery, Wisconsin, were

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between 165–190 mm in total length. We anesthesized all trout with methyl pentynol, measured a sample to the nearest millimeter, and weighed them to the nearest gram. In 1968 and 1969 we weighed, measured, and tagged 106 and 300 trout, respectively, with 1005 controls in 1968 and 500 in 1969. To determine the extent of tag loss, we clipped different fins from the two groups of trout. In 1968 we removed the adipose fin from the control group and the adipose and left ventral fins from tagged groups. In 1969 we removed the adipose and right pectoral fins from the controls and the adipose and right ventrals from the tagged trout.

The tag was 32 mm long, which included 20 mm of yellow, numbered, vinyl tubing. The tagging procedure was described by Dell (1968). We inserted the tags at the posterior base of the dorsal fin. The day following tagging we stocked the trout into Sportsman Lake, Shawano County, Wis.

Sportsman Lake has a surface area of 0.7 ha and a maximum depth of 2.4 m. The pond is fed by ground water seepage with an outlet flow of about 0.2 m^3/sec. The surface waters have an alkalinity of 210 ppm as $CaCO_3$ and a *p*H of 7.6. A continual flow of ground water maintains moderate water temperatures throughout the year. Water temperatures rarely exceeded 18 C at the surface during the summer months while temperatures at 2.0 m rarely exceeded 10 C. The pond was chemically treated in 1967 and a barrier was installed at the outlet to prevent emigration of fish. Recruitment of trout from natural reproduction was negligible during our study.

During both trials we sampled the trout populations four times to measure growth and survival, in winter by ice fishing, and during open water with an a-c boom shocker. We anesthesized, weighed, measured, and applied a temporary finclip to all trout that were captured. Bailey's modification of the Petersen method was used to estimate the number of survivors at each sampling date (Ricker 1958). We determined the confidence limits for the ratio of marked trout in each sample and then calculated the confidence limits for the point estimates (Adams 1951). We estimated the number of survivors in January and March by using the ratio of marked trout in the April or May samples. To obtain population estimates during open-water, we collected trout on successive nights. To test for statistically significant differences in mean weights of each group we used the Student's *t*-test (Steel and Torrie 1960). We calculated instantaneous growth rates using natural logarithms.

Results and discussion — In both trials, control groups grew faster than tagged groups from October to January. In the 1968 trial controls grew 18% faster than tagged trout, though the difference in mean weights between groups was not significant at the 5% level (Table 1). In the 1969 trial the control group grew 28% faster than the tagged group and the difference in mean weights was significant (Table 2). From January to June both groups grew at similar rates and in both trials the differences in growth rates between groups never exceeded 4%. Throughout both trials control groups maintained the weight advantage they acquired during the first months of the experiments. In the 1968 trial the differences in mean weights between groups was only significant in June (Table 1). Six of the ten trout we collected in June 1969, had open wounds at the insertions of the tags.

TABLE 1. Growth and survival of tagged and control groups of brook trout in Sportsman Lake, Wis., 1968–69.

Date	No. sampled	Mean length (*mm*)	Mean weight (*g*)	Instantaneous growth rate per 30 days	Population estimate (95% confidence limits)	Survival (%)
Oct. 23, 1968						
Control					1005	
Tagged	106	178	51		106	
Jan. 15, 1969						
Control	271	196	66	0.092	712(609,853)	71
Tagged	27	196	63	0.075	72(42,169)	68
Mar. 5, 1969						
Control	230	203	75	0.077	662(568,821)	67
Tagged	31	206	72	0.080	62(44,115)	58
May 6, 1969						
Control	306	224	110	0.185	519(417,653)	52
Tagged	23	224	105	0.182	56(25,466)	53
June 16, 1969						
Control	102	239	147[a]	0.212	123(104,161)	12
Tagged	10	234	139	0.205	17(8501)	16

[a]Significantly different at 5% level.

TABLE 2. Growth and survival of tagged and control groups of brook trout in Sportsman Lake, Wis., 1969–70.

Date	No. sampled	Mean length (*mm*)	Mean weight (*g*)	Instantaneous growth rate per 30 days	Population estimate (95% confidence limits)	Survival (%)
Oct. 28, 1969						
Control					500	
Tagged	300	178	51		300	
Jan. 14, 1970						
Control	176	196	64[a]	0.071	453(379,562)	91
Tagged	106	193	60	0.051	268(204,333)	89
Mar. 5, 1970						
Control	189	206	73[a]	0.075	459(399,567)	94
Tagged	102	201	68	0.079	216(176,268)	73
Apr. 23, 1970						
Control	126	211	96[a]	0.168	308(254,410)	64
Tagged	75	208	90	0.172	206(154,309)	69
June 15, 1970						
Control	40	223	115	0.102	57(44,114)	12
Tagged	20	218	108	0.103	36(18,220)	12

[a]Significantly different at 5% level.

These wounds may have caused a decline in growth. In the 1969 trial the control group weighed significantly more than the tagged group from January to April. By June, the difference in mean weights was not significant. Therefore the tags appeared to have initially retarded the growth rate of the trout during the first 3 months and had little effect thereafter.

Survival of the tagged and control groups was similar during the April to June periods for both trials (Tables 1, 2). The control groups had higher calculated survival rates than the tagged groups in March 1969 and 1970. The population estimate in March 1970, is unexpectedly high and we suspect the estimate is in error. About 15 control trout died during handling in January 1970, so the calculated survival figures take into account these losses. Handling mortality occurred when the trout were exposed to air temperatures of –11 to –13 C. No mortality occurred at air temperatures above –11 C. High mortality of trout between May and June in both years was due to angling; the trout fishing season started in mid-May.

We calculated rates of tag loss by dividing the number of trout that lost tags by the total number of different tagged trout that were captured. In the 1968 trial we captured 53 different tagged trout and three had lost their tags for a loss rate of 5.7%. In the 1969 trial we found four trout that lost their tags out of 203 tagged trout, a 2.0% tag loss. The lower rate of tag loss in 1969 trial may be partly the result of more experience in applying the tags.

Throughout both trials the tags remained free of plant growth in the winter months. However, by June periphyton had covered the tags so that the yellow color was hardly visible. Accumulations of filamentous algae also formed on many of the tags, and may have contributed to opening of wounds at the insertions of the tags by causing a fluttering action of the tag as the trout swam about. In June 1969, six of 10 trout examined had open wounds, and in June 1970, two of 20 had wounds.

In summary, the Floy FD-67 anchor tag initially retarded growth of domesticated brook trout but had little effect thereafter. Survival of tagged and control trout was similar and tag loss ranged from 2.0 to 5.7% over a 7-month period. The tendency for algae to accumulate on the tags may limit their usefulness in fertile waters, although we would not anticipate such problems in larger, less fertile lakes.

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Food and Starvation as Factors Affecting the Time to Death of the Lobster *Homarus americanus* Infected with *Gaffkya homari*

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STEWART, J. E., B. M. ZWICKER, B. ARIE, AND G. W. HORNER. 1972. Food and starvation as factors affecting the time to death of the lobster *Homarus americanus* infected with *Gaffkya homari*. J. Fish. Res. Bd. Canada 29: 461–464.

Among lobsters infected experimentally at 15 C with the pathogen *Gaffkya homari*, fed animals died much earlier and with higher bacterial numbers than did animals starved for several months. The shorter times to death were attributed to a more rapid development of the pathogen in the fed host, due to the richer nutrient supply in the circulatory system bolstered by substantial reserves. Infected lobsters ate only during the very early stages of infection.

STEWART, J. E., B. M. ZWICKER, B. ARIE, AND G. W. HORNER. 1972. Food and starvation as factors affecting the time to death of the lobster *Homarus americanus* infected with *Gaffkya homari*. J. Fish. Res. Bd. Canada 29: 461–464.

Parmi les homards infectés expérimentalement à 15 C par la bactérie pathogène *Gaffkya homari*, les individus nourris meurent beaucoup plus tôt et avec une multiplication bactérienne beaucoup plus grande que ceux qui ont jeûné durant plusieurs mois. On attribue cette mortalité hâtive à un développement plus rapide de l'organisme pathogène chez les homards nourris, conséquence d'un apport plus riche de substances nutritives dans le système circulatoire et de la présence de substantielles réserves. Les homards infectés ne mangent que durant les tout premiers stades d'infection.

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A recent review of studies on gaffkemia (Stewart and Rabin 1970), a bacterial infection of lobsters (genus *Homarus*), shows that a major effect of the pathogen's development in vivo is a marked depletion of the host's readily available energy reserves. This depletion was measured (Stewart et al. 1969a) only in animals unfed during the course of the infection. These facts, along with other observations, suggested that the physical condition of infected lobsters, especially the extent of energy reserves or food availability to allow replacement of the energy reserves, might determine the time to death.

The following study reports the mean times to death at 15 C for lobsters (*Homarus americanus*) infected experimentally after being fed and after being starved for 2 or 3 months, the animals' physical condition, and extent of reserves. Data on the lobsters' consumption of food during the infection period was gathered to assess the animals' ability to replenish declining energy reserves.

Methods — The large chelae of each lobster were closed with rubber bands immediately after the animals were captured locally. All lobsters were held at 15 C for a minimum of 2 weeks, and were shown to be free of the pathogen *Gaffkya homari* prior to being included in an experiment. Individual shelters, which the lobsters could use at will, were provided in all tanks. The lobsters were divided into groups, one group to a tank. Some were starved, while others were maintained on a diet that alternated between whole sardine herring and beef liver, given in excess on alternate feeding days. The food was placed in the tanks on Mondays, Wednesdays, and Fridays; surplus food was removed the following day. All experiments were carried out at a constant temperature of 15 C.

The virulence of *G. homari* (Rabin's strain) was maintained by continuous passage through lobsters. For experimental infection purposes, a volume of 0.2 ml o, blood was removed from the infected "passage" lobsterf inoculated into 50-ml Trypticase Soy (TS) broth (Baltimore Biological Laboratory, Baltimore, Md.), and incubated for 24 hr at 28 C. A washed cell suspension in sterile 3% NaCl solution was prepared from this culture and standardized to 0.1 OD (approximately equal to 5×10^6 bacteria/ml) on a Spectronic 20 colorimeter (Bausch and Lomb Inc., Rochester, N.Y.) at 420 mμ, using 12-mm cuvettes. The numbers of viable bacteria were determined by the method of Miles and Misra (1938), using TS agar incubated at 28 C; all dilutions were made with sterile 3% NaCl. Control lobsters were injected with a standard volume (1.0 ml/kg live body weight) of sterile 3% NaCl, and those to be infected were injected with a standard volume of standardized *G. homari* suspensions.

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The injection site was swabbed thoroughly before and after with 70% ethanol.

Protein concentrations in lobster serum were determined (Stewart et al. 1966b) by Robinson and Hogden's modification of the biuret method (Layne 1957); bovine serum albumin (Pro-Sol Standard Scientific Supply Corp., New York, N.Y.) was used as the standard.

Two groups of lobsters, one an uninfected control, the other infected with a standard volume of *G. homari*, were used to determine whether lobsters would eat during the course of the infection. Known weights of food were presented throughout the trial period of approximately 2 weeks. The excess food was removed the following day and weighed; the difference was ascribed to consumption by the lobsters.

Results and discussion — In three trials all of the lobsters deliberately infected died; the differences in the course of the infection attributable to the feeding or starvation regimes lay in the mean times to death. Fed animals died much earlier, with higher bacterial counts at death (Table 1).

The differences in the bacterial counts indicated that the differences in the mean times to death might stem from different rates of bacterial growth in vivo. Difficulties which stemmed from the delicate and sensitive condition of the lobsters, especially during the later stages of the infection when bacterial numbers were high, precluded all but the least amount of physical treatment. This necessitated using large numbers of animals in groups for the construction of a composite curve (Fig. 1). Because of the high numbers of bacteria detected as early as the 4th day in the fed animals, and their obvious sensitivity to handling, the in vivo growth curve was run only for starved lobsters.

Although the uninfected lobsters ate readily and well during the whole trial period, the infected lobsters ate only on the 2nd day after infection, and then only sparingly. Food presented on the 5th and subsequent days following infection was refused thus eliminating the opportunity to replace energy reserves from extraneous sources.

The serum protein values (Table 1), a valid index of condition (Stewart et al. 1967, 1972), clearly show the depletion of reserves in the starved lobsters. The mean bacterial numbers at death were 2–4 times higher, and were reached 2–4 times sooner in the fed groups than in the starved groups. The extent of the contrast between the groups is apparent when terminal bacterial numbers in the fed groups (Table 1) are compared with numbers present at similar times (Fig. 1) in the starved lobsters.

These data are in accord with previous studies (Stewart et al. 1969b) in which it was shown in vitro that the nonprotein nitrogen compounds, notably amino acids, and carbohydrates of the hemolymph were adequate to support the growth of *G. homari*, the extent of the growth being dependent upon the concentration of these materials. A subsequent study (Stewart et al. 1972) has shown that the concentration of nonprotein nitrogen compounds, but not the circulating carbohydrate values, is directly affected by the food supply and its relative value can be assessed using serum protein as the index. Thus, the differences in numbers of *G. homari* in the hemolymph of the fed and starved animals would appear

TABLE 1. Effect of food or starvation on mean time to death of lobsters[a] infected experimentally with *Gaffkya homari* at 15 C.

Date of capture	Number of lobsters	Live wt (*g*)	Prior treatment at 15 C (food)[b]	Serum protein at time of injection (*mg/ml*)	No. of *G. homari* per ml		Time to death (*days*)
					In injection suspension	In hemolymph at death	
May 1968	10	531±20[c]	starved 3 months	not done	7.2×10^6	5.7×10^8	16 ±1.1[c]
	10	578±42	fed 3 months	not done	7.2×10^6	1.9×10^9	3.9±0.3
Oct. 1968	10	298± 8	starved 2 months	23.3±3.2[c]	2.8×10^6	Gram stained hemolymph smears only. Large numbers of tetrads observed; typical of overwhelming infection.	15.1±1.6
	7	256±10	fed 2 months	61.1±4.4	2.8×10^6		8.2±1.8
Mar. 1969	10	570±45	starved 3 months	30.1±3.7	6.4×10^6	5×10^8	14 ±1.6
	9	495±20	fed 3 months	70.1±2.1	6.4×10^6	8×10^8	5.6±0.7

[a]For controls, equivalent numbers of lobsters from the same areas were treated identically except that they were injected with a standard volume of sterile 3% NaCl solution instead of *G. homari* suspensions. None of the controls became infected; only one fed and two starved control lobsters died during the infection trials.

[b]Beef liver and whole sardine herring given in excess on alternate feeding days.

[c]Standard error of the mean.

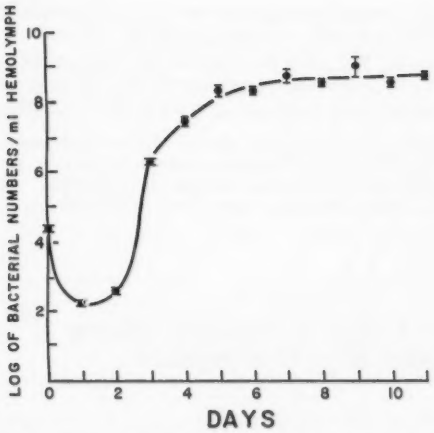

FIG. 1. Composite growth curve of *G. homari* in two groups of 10 lobsters each, starved at 15 C for 3 months. Mean values (and standard errors) given for each group, sampled every 48 hr. The mean serum protein value for all lobsters was 18.4 mg/ml at the beginning of the infection period. After the 3rd day of infection two lobsters died, and subsequent measurements are for two groups of nine lobsters each.

to be a function of the concentration of nonprotein nitrogen, which is high in well-fed animals and low in starved animals. The circulating carbohydrates, notably glucose, play a part and are limiting in vitro, and could be also in vivo when glycogen reserves are low as in starved animals. This would then make the replenishment of circulating glucose a growth-controlling factor. The results and conclusions are consistent with the earlier finding (Cornick and Stewart 1968) that the lobster has no effective intrinsic defenses against an established *G. homari* infection; the rate of *G. homari* growth in vivo would determine the time to death, the rate of growth being controlled by the nutrients available, or being made available.

The mean times to death of lobsters starved for 2–3 months were approximately the same as those for lobsters unfed only during a 2–3 week acclimation period (Table 1, Stewart et al. 1969a). This can be explained by slower development of the bacteria in hemolymph lower in essential nutrients. The decline in physical condition of lobsters unfed at 15 C is relatively rapid, with significant differences apparent even within a 2–3 week period (Stewart et al. 1972; Stewart et al. 1969a), thus making the hemolymph a poorer source of nutrients for *G. homari* and extending the period required to develop bacteria in numbers large enough to cause the death of the lobster.

The lack of feeding by lobsters after the development of relatively large numbers of *G. homari* should affect captures of infected lobsters at sea. An infected lobster would not be attracted by the bait in the traps except during the very early stages of the infection. This is consistent with the results of field surveys reported previously (Rabin 1965; Stewart et al. 1966a).

The results of our studies suggest reasons why the epizootics in commercial holding units, especially those with newly captured lobsters, can occur so abruptly and with such devastating results. Freshly captured lobsters, except for the short time period following molting (Stewart and Li 1969), would have relatively high serum protein values, and from that can be assumed to have relatively high nonprotein nitrogen values. This fact, in addition to the often extremely adverse conditions of captivity, including those promoting widespread transmission of the disease (Stewart and Rabin 1970), would effect a shorter mean time to death among infected lobsters than times recorded in laboratory experiments.

Acknowledgments — The authors thank Dr R. A. Laycock for her constructive criticism of the manuscript.

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Relation of Egg Number to Carapace Length of Sidestripe Shrimp (*Pandalopsis dispar*) from Dabob Bay, Washington[1,2]

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HARRIS, C., K. K. CHEW, AND V. PRICE. 1972. Relation of egg number to carapace length of sidestripe shrimp (*Pandalopsis dispar*) from Dabob Bay, Washington. J. Fish. Res. Bd. Canada 29: 464–465.

A linear relation was found between egg number and carapace length in a sample of 244 sidestripe shrimp, *Pandalopsis dispar*. There was an average error of 6% in estimates of egg number. Ovigerous sidestripe shrimp from Dabob Bay are smaller than those of Vancouver Island, and have lower egg counts than those of Alaska.

HARRIS, C., K. K. CHEW, AND V. PRICE. 1972. Relation of egg number to carapace length of sidestripe shrimp (*Pandalopsis dispar*) from Dabob Bay, Washington. J. Fish. Res. Bd. Canada 29: 464–465.

Nous avons démontré une relation linéaire entre le nombre d'oeufs et la longueur de la carapace sur un échantillon de 244 crevettes, *Pandalopsis dispar*. L'erreur moyenne d'estimation du nombre d'oeufs a été de 6%. Les crevettes ovigères de cette espèce de la baie Dabob sont plus petites que celles de l'île de Vancouver et ont moins d'oeufs que celles de l'Alaska.

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INFORMATION on eggs carried by sidestripe shrimp (*Pandalopsis dispar* Rathbun) is scarce. Berkeley (1930) and Butler (1964) made little mention of egg number in discussions of its postembryonic life history. Hynes (1930) reported an average egg count of 4150 for a sample of 25 ovigerous females. This study was made to determine the relation between egg number and carapace length.

Materials and methods — In January 1971, 244 ovigerous females were caught with a 40-ft semiballoon shrimp trawl in Dabob Bay, Wash. Egg masses were removed and a sample of 100 eggs was weighed to an accuracy of 0.1 mg. The entire egg mass was then weighed and the total number of eggs estimated. Egg size varied considerably among individuals. Stage of development differed also. Carapace length was measured to the nearest 0.5 mm from the dorso-posterior end to the base of the eye socket.

Results and discussion — The data on egg numbers are plotted against the carapace length measurements in Fig. 1. Some shrimp had considerably fewer eggs than most of the others, although they had the same carapace length. Variation probably is due to losses during netting or in subsequent handling. However, the low counts of eggs obtained for some

[1]Contribution No. 395, College of Fisheries, University of Washington.

[2]The procurement of shrimp for this study was made possible by NSF Grant GH-40, under the Sea Grant Program. The Sea Grant Program is now maintained under NOAA, U.S. Department of Commerce.

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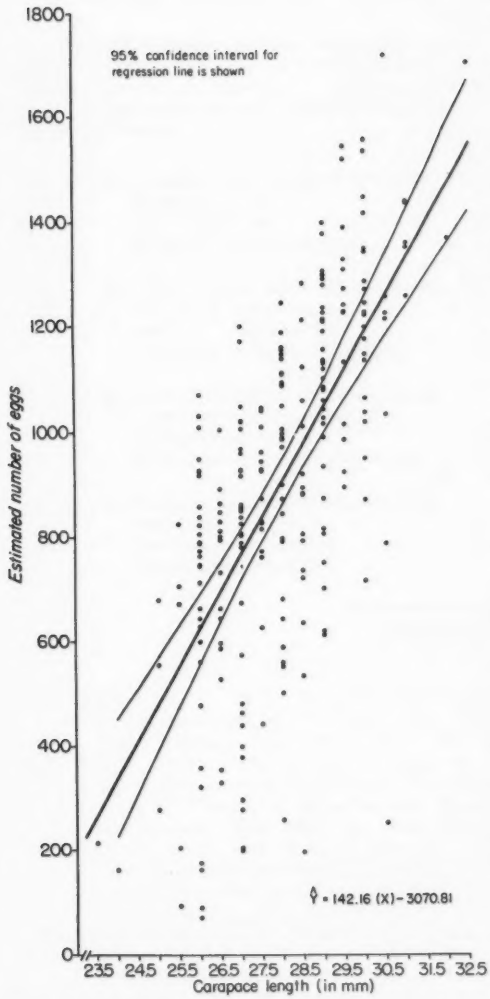

FIG. 1. Relation between egg numbers and carapace length in the entire sample.

shrimp probably did reflect a low abundance of eggs at the time of capture. Throughout the period of incubation there is bound to be a continued attrition of eggs due to dislodgement or mortality with subsequent disappearance. Such factors of attrition had been operating on the egg masses of the sampled shrimp for at least 3 months before the shrimp were collected in January. This may account for the wide spread of counts obtained in this study. Despite the wide distribution of points in Fig. 1, there was a linear relation between estimated number of eggs and carapace length ($\hat{Y} = 142.16(X) - 3070.81$).

Very important to this study is the accuracy of the method of estimating egg number. Actual counts of eggs (326–939), as well as estimates (332–1072), were carried out on 15 females. Errors, mainly towards overestimation, were from 2 to 15%, and averaged 6%. Factors contributing to error may have been: (1) an excessive amount of water on the eggs, (2) debris in the egg mass, and (3) breakage and loss of contents of eggs in the sample of 100. The last factor was probably the major source of error, as recognizing and accounting for broken eggs were difficult.

Besides just showing a relation between egg numbers and carapace length, this study suggests that the Dabob Bay sidestripe shrimp may be significantly smaller and have less eggs than those of more northern waters. Hynes (1930) found an average egg count of 4,150 in his sample from Alaska. The shrimp in this study had a mean number of only 904 eggs. Butler (1964), working with a sample from Vancouver Island, found that females aged 34 months had a mean carapace length of about 30 mm (range 24–33 mm). The shrimp in this study (of approximately the same age) had a mean carapace length of about 28 mm (range 22–32.5 mm). This mean and range are more comparable to those for Vancouver Island shrimp in the transitional stage, aged about 26 months. The fact that Dabob Bay is near the extreme southern part of the range of this species might explain these differences in egg numbers and size.

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Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

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Abstracts should be provided in both English and French.

The *Style Manual for Biological Journals* (2nd ed. American Institute of Biological Sciences, 2000 P St. NW., Washington, D.C.) is, with few exceptions, used as the authority in matters of form. *Webster's Third New International Dictionary* is the standard for spelling and division of words in English; *le Grand Larousse Encyclopédique*, for those in French. Exceptions are made for manuscripts that follow the *Oxford English Dictionary* entirely. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. Manuscript reports are included in the list of references, but are identified both there and in the text by the letters "MS" before the year. The *Journal* follows the spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970), Special Publication No. 6 of the American Fisheries Society.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued before completion; or on preliminary findings that warrant publication before problems are resolved satisfactorily. Or they may be used to establish priority in publication. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

In general, *series titles* should be avoided.

Illustrations

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and legible when the illustration is reduced to the appropriate size. Drawings should be grouped, when feasible, for economy of space. Originals must be submitted and should be less than 20 inches in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Prints of photographs should be made on glossy paper with a wide range in tone. They should be cropped, squared, and mounted with rubber cement on white cardboard, with no space between those arranged in groups.

Proofs and Reprints

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty reprints, without covers, are supplied free. Additional copies needed must be ordered, on the form provided, when the galley proof is returned.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors. The same questions are recommended to referees who review manuscripts for the *Journal*.

General

Is there new knowledge in the report worth publishing?
Are the findings, interpretations, and conclusions sound, and relevant to the purpose of the study?
If the manuscript is merely confirmatory, is it worth publishing?

Does the manuscript need to be rewritten before the work can be assessed?

Is the contribution placed in the proper perspective concerning the state of knowledge of the subject?

Is the manuscript too long?
Are all the tables and figures needed, and organized for economy of space?
Would any of the text be clearer if condensed?
Should some of the data be made available separately, in a manuscript report or at a data repository?
Should the manuscript be condensed as a Note?

Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics?

Are there inconsistencies between tables or figures and the text, or within the text?

If the manuscript is from a thesis, does it retain characteristics common in these but not desirable in journals?

Parts of the Manuscript

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Has the *introduction* all the important guideposts for orienting the reader?

Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data obtained?

Are generalizations on the *results* adequate?

Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

If there are loopholes in interpretation, are they acknowledged? Should they be overcome?

Should other interpretations be recognized?

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Guide des auteurs

Des détails sur le tapage et certains autres item sont donnés dans le "Guide pour les secrétaires d'auteurs." Aussi, une liste d'épellations recommandées d'environ 100 mots relatifs aux articles sur les pêcheries est disponible au bureau du Directeur de la publication.

Disposition générale

Les résumés doivent être fournis en anglais et en français.

Le *Style Manual for Biological Journals* (deuxième édition, publié par l'American Institute of Biological Sciences, 2000, rue *P* N.-O., Washington, D.C.), a été adopté comme guide des auteurs. Le *Third New International Dictionary* de Webster, dans le cas de l'anglais, et le *Grand Larousse Encyclopédique*, dans le cas du français, font respectivement autorité pour l'orthographe et la séparation des mots. Une exception est faite à l'égard des travaux en anglais qui se conforment entièrement à l'*Oxford English Dictionary*. Les titres de périodiques sont abrégés selon la *Biological Abstracts*. Toute référence à la littérature doit suivre le système nom-année. Les rapports manuscrits sont inclus dans la liste des références, les lettres "MS" avant l'année les identifient dans les références et dans le texte. Le *Journal* se sert des épellations recommandées dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No 6 de l'American Fisheries Society.

Les *notes* se distinguent des écrits d'après l'étendue de la recherche plutôt que par la qualité de la recherche. Les notes peuvent être sur des travaux de recherche de portée restreinte, susceptibles de découler d'investigations plus considérables, pour la description de techniques de recherche et de méthodes de pêche, pour l'établissement de priorités de publication, et pour faire connaître les renseignements utiles tirés d'entreprises qui ont été interrompues avant d'avoir été achevées. Si la portée de la Note le permet, elle peut être disposée comme un mémoire. D'autre part, la disposition est flexible. La longueur ne doit pas dépasser quatre pages imprimées.

En général, les mémoires en série doivent être évités.

Les illustrations

Les dessins au trait doivent être exécutés à l'encre de chine sur papier à dessin blanc ou sur papier à calquer, ou sur papier à coordonnées avec quadrillage bleu. Les lignes, les symboles, les lettres et les chiffres doivent tous être propres. Ils doivent être lisibles quand ils sont réduits à la grandeur voulue pour la reproduction désirée. Les dessins doivent être groupés, si la chose est possible, de façon à utiliser tout l'espace disponible. Les originaux doivent être soumis et devraient mesurer moins de 20 pouces dans le sens le plus long pour les illustrations de pages entières, et proportionnellement plus petit pour les illustrations de moins d'une page. Les dessins doivent être postés à plat.

Les épreuves des photographies doivent être tirées sur papier glacé et accuser une gamme étendue de teintes. Elles doivent être coupées, dressées et montées, au moyen de dissolution, sur carton blanc, sans laisser d'espace entre celles qui sont disposées par groupes.

Epreuves et tires a part

Les épreuves en placards sont adressées à l'auteur et la responsabilité de toute erreur qui peut y rester lui incombe.

Cinquante tirés à part, sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander d'autres tirés à part.

Liste de contrôles pour manuscrits

Une attention particulière portée sur les questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs. Les mêmes questions sont recommandées aux arbitres qui font la critique des livres pour le *Journal*.

Generalites

Est-ce que les connaissances nouvelles du rapport méritent d'être publiées?

Les découvertes, les interprétations et les conclusions sont-elles solides et pertinentes au but de l'étude?

Si le manuscrit est tout simplement corroboratif, mérite-t-il d'être publié?

Le manuscrit doit-il être récrit avant d'évaluer le travail?

La contribution est-elle placée dans une perspective appropriée relative à l'état des connaissances du sujet?

Le manuscrit est-il trop long?

Les tables et figures sont-elles toutes nécessaires et arrangées pour économiser le plus d'espace?

Le texte serait-il plus clair s'il était condensé?

Est-ce qu'une partie des données devrait être disponible séparément, soit dans un rapport ou dans un dépôt des données?

Le manuscrit devrait-il être condensé en une note?

Est-ce que la préparation du manuscrit suit la logique mentionnée dans l'introduction?

Y a-t-il des exposés sommaires au début de chaque section et paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents aux sujets?

Y a-t-il des contradictions entre les tableaux et les figures du texte, ou dans le texte même?

Si le manuscrit est tiré d'une thèse, contient-il des caractéristiques communes à cette dernière, mais indésirables dans un journal?

PARTIES DU MANUSCRIT

Le titre renferme-t-il le contenu du rapport?

Le résumé donne-t-il l'essentiel des connaissances nouvelles?

L'introduction a-t-elle tous les points importants afin d'orienter le lecteur?

Le renvoi bibliographique se limite-t-il à la définition du problème?

Les détails du matériel et des méthodes employées sont-ils limités à un minimum pour que les scientifiques comprennent le but de l'étude et puissent porter un jugement adéquat sur les données obtenues.

Les généralisations sur les résultats sont-elles adéquates?

Les conclusions sont-elles déduites des résultats?

La discussion est-elle limitée à l'interprétation et la signification de ces découvertes?

S'il y a des échappatoires dans l'interprétation, sont-elles reconnues? Devraient-elles être maîtrisées?

Devrait-on accepter d'autres interprétations?

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Guide for Typists Preparing Copy

Read the "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 8.5 × 11 inches (21.5 × 28 cm). If possible, use paper with the lines numbered in the margin. Leave all margins at least 1 inch (2.5 cm) wide. Use a nylon or carbon ribbon and keep the typewriter keys clean.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numbers. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (Appendix A). Type the abstract on a separate page (Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send two copies (the ribbon copy and a carbon copy or a photocopy) to the Office of the Editor.

Tables

Examine the current volume of the *Journal* to see how tables are set up (Appendix C).

Note especially the following concerning *numbered tables*:

Type each table on a separate page, using paper without the lines numbered.

Number the tables with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, they are typed with the text, they seldom need horizontal lines, and the data are arranged in as few rows as possible (Appendix C).

Illustrations

Send the original and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas

Leave three spaces above and below lines that have much material in superscripts or subscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral one, and between the capital letter O and the numeral zero. Make other explanatory notes similarly.

References

Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style that is used in the *Journal* (Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list, but if an abbreviation is in doubt, type the name in full.

Spellings

A list of about 100 words common in fisheries journals is given in Appendix F.

Appendices

Appendices A to F are available on request from the Office of the Editor.

Journal Fisheries Research Board Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des auteurs" dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de taper ou d'arranger les détails, suivez la pratique du *Journal*.

Tapez le manuscrit sur un papier blanc et non un papier pelure, mesurant de préférence 8½ × 11 po. (21.5 × 28 cm) et laissant une marge d'au moins 1 po. (2.5 cm). Si possible, employez un papier avec des lignes numérotées dans la marge. Employez un ruban nylon ou carbone et gardez les clés du dactylo nettes.

Chaque partie du manuscrit doit être tapée à double-interligne au moins. La chose est particulièrement importante dans le cas des tableaux, légendes d'illustrations, renvois au bas de la page et listes de références bibliographiques. Evitez de souligner à moins que la matière ne soit en italique. Mettez en retrait la première ligne de chaque paragraphe du texte, ainsi que de chaque légende et renvoi.

Dans les titres et en-têtes, n'employez la majuscule que pour le premier mot, les noms propres et les adjectifs. Pour les petites majuscules, utilisez les petites lettres.

Indiquez tous les renvois au texte par des chiffres arabes en-tête. Numérotez-les consécutivement à travers le texte en commençant par ceux de la page-titre. Placez chaque renvoi immédiatement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit donner que le titre du sujet, le nom et l'affiliation de l'auteur ou des auteurs, et tout autre renvoi utile (Appendice A). Tapez le résumé sur une page séparée (Appendice B). Numérotez chaque page, débutant avec la page-titre.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et un double du travail au Directeur de la publication.

Tableaux

Consultez une copie du *Journal* pour savoir composer les tableaux et placer les lignes qui y entrent (Appendice C).

Portez une attention particulière à ce qui suit concernant les *tableaux numérotés*.

Tapez les tableaux sur des pages séparées, en utilisant du papier sans lignes numérotées.

Numérotez les tableaux avec des chiffres arabes.

Employez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, et très rarement ailleurs. N'employez jamais de lignes verticales; de préférence, laissez de l'espace supplémentaire.

Indiquez les renvois dans les tableaux au moyen de petites lettres italiques en position supérieure et tapez-les au bas de la page.

Placez les tableaux après la liste de références.

A l'endroit où un tableau est mentionné pour la première fois, tapez dans la marge "Tableau (- - -) près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, ils sont tapés avec le texte. Ils ont rarement besoin de lignes horizontales, et l'information est préparée de façons à prendre le moins de rangées possibles.

Illustrations

Joignez au manuscrit expédié le dessin original des illustrations et des photocopies en deux exemplaires.

Au bas et à gauche de chaque figure et photocopie, inscrivez le nom de l'auteur et le numéro de la figure. Aussi, tapez chaque légende avec sa figure pour fin d'identification. Pour les photographies, tapez l'information sur une lisière de papier et collez-la au dos de la photographie. N'écrivez pas et ne tapez pas sur la photographie même. Tapez ensemble sur une feuille séparée et par ordre consécutif les légendes des illustrations, et intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, tapez dans la marge "Fig. (- - -) près d'ici."

Equations, formules et expressions mathematiques

Laissez trois espaces au-dessus et au-dessous des lignes qui contiennent du matériel en positions supérieures ou en positions inférieures, et au-dessus et au-dessous d'équations et de formules. Dans les différentes expressions, placez chaque composante dans sa position appropriée (Appendice D).

Indiquez les symboles clairement. Dans la marge, épelez les lettres grecques la première fois qu'elles apparaissent et faites une distinction entre la lettre "l" et le chiffre "un" et entre la majuscule "O" et le

chiffre "zéro." Procédez de la même façon pour d'autres notes explicatives.

References

Vérifiez le texte afin d'assurer que sont mentionnées toutes les références ou renvois de la liste. Tapez, à doubles interlignes et de la façon prévue (Appendice E) pour le *Journal*, la liste de références. Les noms de journaux sont abrégés suivant la *Biological Abstracts*, mais s'il y a doute, écrivez le nom au long.

Vocabulaire

Une liste d'environ cent mots relatifs aux articles sur les pêcheries est disponible au bureau du Directeur de la publication (Appendice F).

Appendices

Les appendices de A à F sont disponibles sur demande au bureau du Directeur de la publication.

Periodical Publications

of the

American Fisheries Society

Organized in 1870, the American Fisheries Society is the oldest professional body in North America dealing with fish and fisheries resources.

The *Transactions*, the Society's official organ published since 1872, is a quarterly journal of fisheries science and technology. Its authoritative articles promote the conservation of the aquatic environment and influence many of the major advancements in fisheries management. Volume 100 (1971) contains 830 pages.

Annual subscription: Libraries, $25 (includes special publications issued during the year); active members, $20.

The Society also publishes English-language editions of two Russian bimonthly journals:

Hydrobiological Journal

It reports on studies in the biochemical, biophysical, and ecological properties of the world's fresh and salt waters.

It is of interest to North American limnologists, hydrologists, oceanologists, and marine specialists.

Annual subscription (6 issues): $58

Journal of Ichthyology

It reports the findings of Russian investigators in all aspects of freshwater and saltwater biology.

It is a serviceable reference source for fisheries biologists, particularly research workers, students, and teachers.

Annual subscription (6 issues): $78

These journals may be ordered from:

American Fisheries Society
1040 Washington Building
New York Avenue at 15th Street, N.W.
Washington D.C. 20005
U.S.A.

Fisheries Research Board of Canada

Bulletins have a new look!

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A synopsis of Canadian marine zooplankton

C.-T. Shih, A. J. G. Figueira, E. H. Grainger
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paralytic shellfish poisoning in eastern Canada

A. Prakash, J. C. Medcof, A. D. Tennant
(vii + 87 p., 1971, $3.00)

Bulletin 178

Pacific oyster raft culture in British Columbia

D. B. Quayle
(viii + 34 p., 1971, $2.00)

These three new numbers in the series are available at the prices shown from:

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Journal of the Fisheries Research Board of Canada

Volume 29, No. 4, April 1972

JFRBCA29(4)349–468(1972)

Residues of organochlorine pesticides and polychlorinated biphenyls in some commercially produced Canadian marine oils — R. F. Addison, M. E. Zinck, and R. G. Ackman — 349–355

Some relations of land drainage, nutrients, particulate material, and fish catch in two eastern Canadian bays — W. H. Sutcliffe Jr. — 357–362

Review of studies on feeding of aquatic invertebrates conducted at the Institute of Biology of Inland Waters, Academy of Sciences, USSR — A. V. Monakov — 363–383

Molluscan sterols: a review — D. R. Idler and P. Wiseman — 385–398

Physiological responses of three species of fishes to various salinities — R. W. Brocksen and R. E. Cole — 399–405

Lipid composition of the queen crab (*Chionoecetes opilio*) — R. F. Addison, R. G. Ackman, and J. Hingley — 407–411

Laboratory rearing of spot shrimp larvae (*Pandalus platyceros*) and descriptions of stages — V. A. Price and K. K. Chew — 413–422

Size at maturity in the male Alaskan tanner crab, *Chionoecetes bairdi*, as determined by chela allometry, reproductive tract weights, and size of precopulatory males — R. B. Brown and G. C. Powell — 423–427

Bacterial population of diets for aquarium fishes — T. J. Trust and V. G. Money — 429–433

Effect of salmon gonadotropin on in vitro maturation of oocytes of a catfish, *Heteropneustes fossilis* — B. I. Sundararaj, S. V. Goswami, and E. M. Donaldson — 435–437

Effects of temperature, food, and starvation on several physiological parameters of the lobster *Homarus americanus* — J. E. Stewart, G. W. Horner, and B. Arie — 439–442

Clostridium botulinum in sediments from the Canadian Atlantic seaboard — R. A. Laycock and A. A. Longard — 443–446

Notes

Mating behavior in the spider crab, *Chionoecetes opilio* — J. Watson — 447–449

Distribution of iron, magnesium, copper, zinc, and silver in oysters along the Georgia coast — H. L. Windom and R. G. Smith — 450–452

Aging round whitefish (*Prosopium cylindraceum*) of the Leaf River, Ungava, Quebec, by otoliths — B. M. Jessop — 452–454

Marking larval largemouth bass with radiostrontium — C. A. Carlson and M. H. Shealy Jr. — 455–458

Effects of the Floy anchor tag on the growth and survival of brook trout (*Salvelinus fontinalis*) — R. F. Carline and O. M. Brynildson — 458–460

Food and starvation as factors affecting the time to death of the lobster *Homarus americanus* infected with *Gaffkya homari* — J. E. Stewart, B. M. Zwicker, B. Arie, and G. W. Horner — 461–464

Relation of egg number to carapace length of sidestripe shrimp (*Pandalopsis dispar*) from Dabob Bay, Washington — C. Harris, K. K. Chew, and V. Price — 464–465

Recent articles on FRB investigations published in other periodicals — 467

Additional FRB publications — 468

Guide to Authors and Typists — *i–vii*

INFORMATION CANADA, OTTAWA, 1972